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THE INTERNAL TEMPERATURES OF FRUIT-TREE BUDS. II

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(With 10 Text-figures)

CONTENTS

INTRODUCTION

THE present paper amplifies and extends a previous investigation (Grainger & Allen, 1936) where temperatures within dormant buds of apple, black currant and raspberry were measured by thermo-electric apparatus.

METHODS

A circuit of two fine iron-constantan thermocouples connected in series was used to make continuous measurements of temperature differences at the junctions upon a Cambridge thread-recording galvanometer. Details were given in a previous paper (Grainger & Allen, 1936), and the records show actual air temperatures, but only compare internal bud temperatures with those of the surrounding air. Atmospheric temperatures were occasionally measured by a standard bimetallic thermograph made by Messrs Negretti and Zambra. This was placed near to the buds under investigation, and was enclosed within a louvre screen $18 \times 15 \times 14$ in. high. Thermo-electric circuits were further modified, in some of the experiments here described, to estimate solar radiation (Fig. 1) and "wet-bulb" temperatures (Fig. 2). Curves representing solar radiation occasionally went below zero on the chart at night, indicating radiation from the apparatus to an open sky (lowest curve, Fig. 9).

Two records were sometimes made upon each chart of the galvanometer, and were separated by a mechanical method. Two inked threads of contrasting colour were brought alternately below the recording mechanism, whilst a two-way switch changed

the circuits. Each circuit was thus always recorded by a particular colour, and the circuits were chosen for their contrast. Air temperatures and bud temperatures contrasted well, or buds with different temperature responses, such as apple and raspberry. Both curves on one chart had the same zero, which was marked by the usual method of disconnecting the common lead to the galvanometer for a few minutes each day.
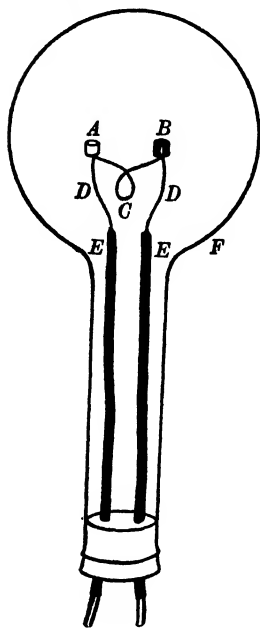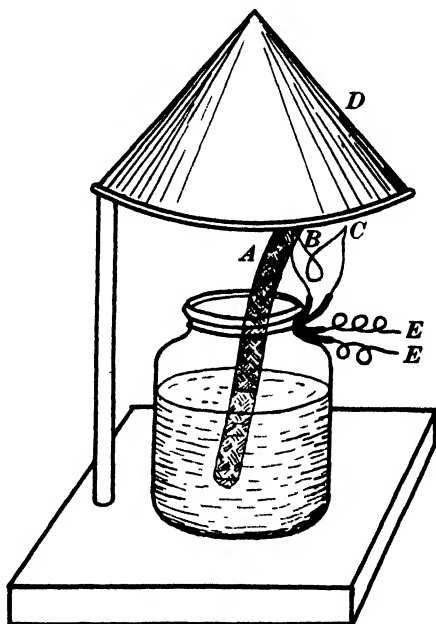
Fig. 1.   Fig. 2.

Fig. 1. Apparatus for the electrical measurement of solar radiation. *A*, a bright cylinder of brass; *B*, a dark cylinder; *C*, constantan wire; *D*, iron wire; *E*, leads of insulated copper wire; *F*, flask of Pyrex glass. Cylinder *A* reflected some of the sun's heat, whilst *B* absorbed it, thus giving a temperature difference which was recorded.

Fig. 2. Apparatus for the electrical measurement of humidity. *A*, wick dipping into water in the bottle; *B*, thermocouple inserted into the wick; *C*, thermocouple in the air; *D*, hood; *E*, leads of insulated copper wire.

RESULTS

The following results are selected from a total of 594 daily records made in 1936, 1937 and 1938.

The effect of frosts upon apple-bud temperatures

Fourteen records of frosts during the dormant period were obtained in 1936 and 1937. They exhibit similar features to six more which were obtained in the earlier investigations in 1932 and 1933 (Grainger & Allen,

1936), in that the bud temperature coincided fairly closely with the air temperature during the more severe part of the frost. Fig. 3, which is typical, shows this from 1 a.m. to 6 a.m. The rise in the relative bud temperature between 6 a.m. and 7 a.m. was due to the warming effects of the sun's radiation, which first reached the bud soon after 6 a.m. A fall in the bud temperature relative to that of the air followed between 7 a.m. and 8 a.m., but this does not represent a fall in the *actual* temperature of the bud. This was increasing all the time from 6.30 a.m. to 8.30 a.m., as shown by the dotted line in the upper curve of Fig. 3.
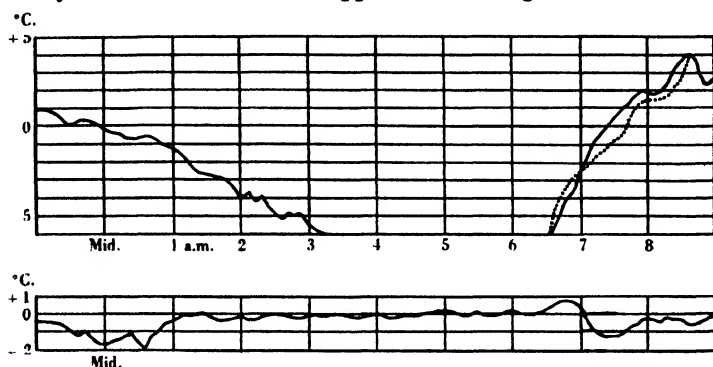
Fig. 3. A frost during dormancy of the apple bud. Upper curve, air temperature; lower curve, temperature of the bud in relation to the surrounding air. The actual temperature of the bud is shown by the dotted line in the upper diagram, between 6.30 a.m. and 8.30 a.m. 5–6 March 1937.

The early stages of the frost shown in Fig. 3 are marked by a rise in the temperature of the bud relative to the surrounding air between midnight and 1 a.m. This is somewhat comparable to the behaviour of opening buds to frosts (Grainger & Allen, 1936). Three more such records were obtained when buds were bursting in 1938, and Fig. 4 shows a typical example. The bud temperature approximated to that of the surrounding air until the latter reached freezing-point, but became relatively warmer when the air temperature fell slightly below that point, i.e. after midnight. This is the "frost compensation mechanism" (Grainger & Allen, 1936), but it is now obvious that it is not of great magnitude, and occurs only during slight frosts of 1 or 2° C., or at the commencement of more severe frosts. Though it is a frequent phenomenon, it cannot be of much practical significance.

It is to be expected that buds would have a different composition when they are opening from when they are dormant. The actual freezing-

points of standardized extracts were determined in 1937. Apple buds were not available in sufficiently large quantities, so hazel, oak and sallow (*Salix caprea*) were used. It is impracticable to obtain expressed sap from dormant buds unless inordinately large quantities are available, so a standardized extract was made with distilled water.
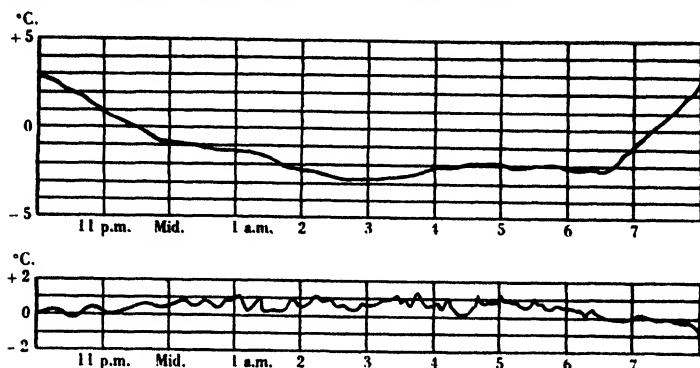
Fig. 4. A frost when buds of the apple (var. Allington Pippin) were bursting. Above, air temperature (bimetallic record). Below, temperature of the bud in relation to the surrounding air. 7–8 May 1938.

Freezing-points of such extracts were compared, immediately after their preparation, with the freezing-point of distilled water, by means of a Beckman thermometer. The percentages of water, of organic matter, and of ash were determined for portions of each sample of buds, and the differences recorded in the last two columns of Table I are of sufficient magnitude to be significant with relatively large variations in water content.

Table I. *Comparative estimations of standardized extracts from buds at various stages*

Tree	Condition of buds	Date	% water	% organic matter	% ash	Depression of freezing-point in °C.	Osmotic pressure in atm.
Hazel	Dormant	6. i	50·00	48·50	1·50	0·15	1·805
Hazel	Opening	19. iii	55·25	43·08	1·62	0·38	4·580
Hazel	Young leaves	5. v	71·40	25·83	1·46	0·11	1·327
Oak	Dormant	19. iii	45·52	52·00	1·98	0·13	1·568
Oak	Opening	5. v	69·20	28·46	2·31	0·17	2·050
Sallow	Dormant	19. iii	47·18	52·00	0·08	0·04	0·398
Sallow	Opening	5. v	71·54	26·51	1·74	0·10	1·206

The depression of the freezing-point of standardized extract is greatest at the time of opening of the buds. It is at this time also that the greatest

damage by frost can occur. Such damage, therefore, cannot be caused by any increased tendency of the sap to congeal at the time of opening, and other explanations must be sought.

The effect of frost control by orchard heaters upon bud temperatures

A practicable method of frost control by inexpensive heaters which burn crude oil has been introduced by Mr G. Harrington.[1] The question as to whether the heaters merely raised the temperature of the air, or heated the buds by direct radiation from the flames, was tested by ten trials in February 1937, and April and May 1938.
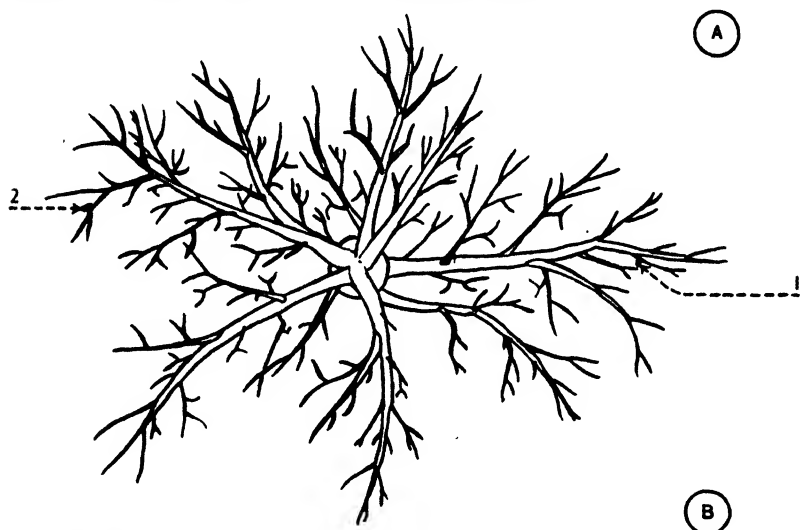
Fig. 5. Sketch plan of apple tree (var. Lord Suffield), showing position of the orchard heaters, A, B, and buds, 1, 2. Bud 1 could be heated by direct radiation from the flames of heater B, but was screened by the branch from A. Bud 2 was 8 ft. above the ground, and was not apparently affected by either of the heaters. 23 Feb. 1937.

Estimations during a radiation frost on the evening of 23 February 1937 provided typical results. Fig. 5 shows the arrangement of heaters round an apple tree of the variety Lord Suffield. Internal temperatures of two buds, 1 and 2 in the figure, were recorded, whilst air temperatures were measured by a screened thermograph placed close to bud 1, and by mercury thermometers round bud 2, and in the unheated parts of the orchard. Bud 1 was 2 ft. 6 in. above the ground, and 5 ft. from either

[1] Marketed by Messrs Geo. Monro, Ltd., of Waltham Cross.

heater; bud 2 was 8 ft. above the ground, and 16 ft. from either heater. The latter bud was quite unaffected by the heaters, for no difference could be found between temperatures of the air surrounding it and temperatures in the unheated parts of the orchard (see dotted line in I, Fig. 6). The apparent rise in the internal temperature of this bud (II in Fig. 6) is occasionally found with falling air temperature, and may be
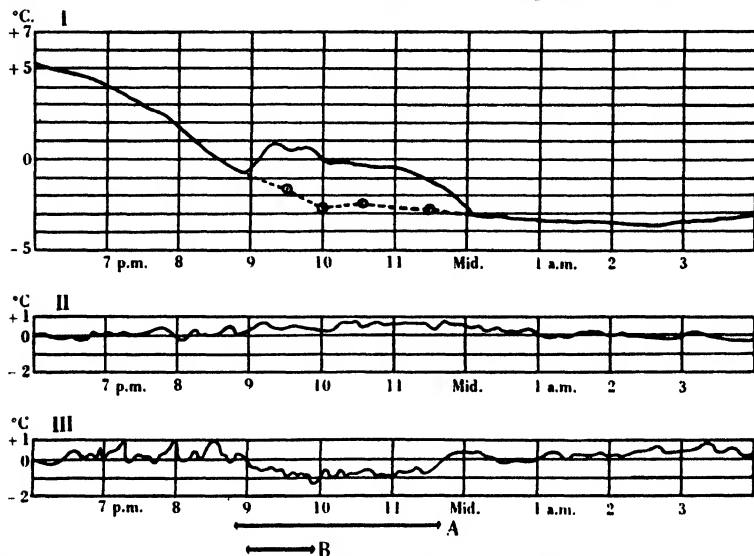
Fig. 6. I. Raising of the air temperature by orchard heaters 8.45 p.m. to 11.40 p.m.; bi-metallic record. Temperatures of the unheated air in the orchard are shown by the dotted line. II. Temperature (in relation to the surrounding air) of bud 2 (fig. 5) not affected by the heaters. III. Temperature of bud 1 (fig. 5) in relation to the surrounding air. A = length of time heater A was lit, namely 8.45 p.m. to 11.40 p.m. B = length of time heater B was lit, namely 9 p.m. to 9.50 p.m. Heater A could not heat bud 1 (curve III above) by direct radiation; heater B could possibly do so. 23 Feb. 1937.

due to delay of the bud in assuming the temperature of its surroundings. It gives the further proof of having no connexion with the heaters, that it continues for 1½ hr. after they are extinguished. Heating of the orchard under commercial conditions would, of course, have brought bud 2 within the sphere of two further heaters.

The greatest increase in air temperature accomplished by the heaters in any of the ten trials was only 3° C. (5·5° F.) when the nearest heater was 5 ft. from the thermograph. This only happened, moreover, when the air was still, or when a slight, downwardly flowing katabatic wind

(Cornford, 1937) impelled the warm air towards the thermograph. A rise of 2° C. (4° F.) was the usual increase in still air, but any greater distance than 5 ft. from heater to tree or thermograph quickly reduced the rise in temperature.

One heater (B in Fig. 5) was so disposed that it could possibly heat bud 1 by direct radiation, whilst the flames from the other heater (A) were shielded from the bud by a thick branch. Curve III in Fig. 6 shows the results, and indicates no difference between the effect of either heater or both. This means that the heaters exert their good effect by raising the temperature of the air, and not by direct radiation from the flames. The temperature of the bud was uniformly *lower* than the temperature of the
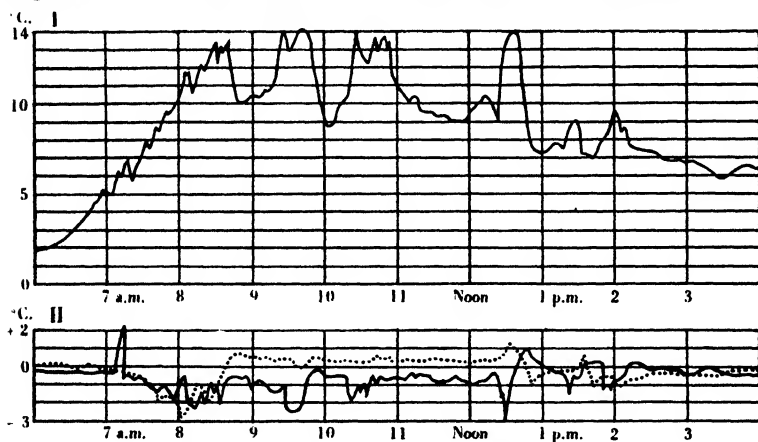
Fig. 7. 1. Air temperature. II. Temperatures, in relation to the surrounding air, of two adjacent apple buds (var. Bramley's Seedling), 4 in. apart upon the same branch. 9 Oct. 1936.

surrounding air, owing to the effect of the heaters in decreasing the relative humidity of the air. Evaporation would thereby be increased, and the bud's temperature would therefore be lowered. Since the temperature inside a bud is lowered by heaters which only raise the air temperature 2 or 3° C., the margin of efficiency of the flame-type heaters would seem to be narrow. This aspect of the investigations is discussed more fully later in this paper.

Differences in temperature response between two adjacent apple buds

There is usually a strong agreement between the temperature reactions of two adjacent apple flower buds. Thirty-one pairs of records show such agreement, but Fig. 7, curve II, shows an instance where different

responses were observed between two flower buds separated by only 4 in. from each other. These two curves are typical of ten pairs of records. There is occasionally a qualitative similarity of the curves, but sufficient dissimilarity in detail to show some individuality of response by different buds exposed to approximately the same environment.

Temperature responses of the raspberry

Estimations of the temperatures of dormant raspberry buds, of opening raspberry flowers, of flowers and of fruits, were made upon the variety Lloyd George in 1936. Forty curves relating to the dormant
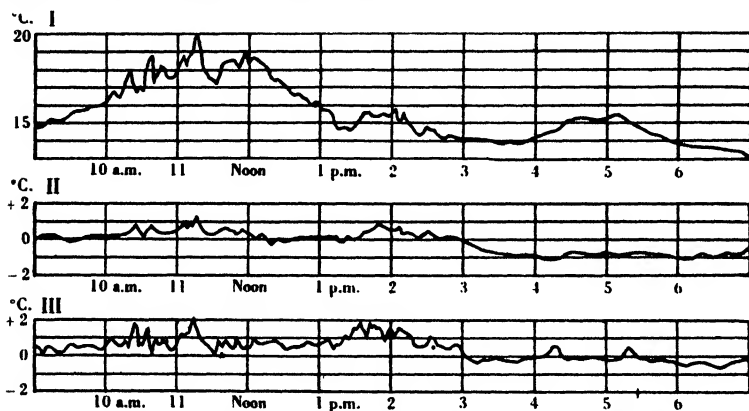
Fig. 8. I. Air temperature. II. Temperature of dormant apple bud (var. Bramley's Seedling) in relation to surrounding air. III. Temperature of dormant raspberry bud (var. Lloyd George) in relation to surrounding air. 17 Oct. 1936.

period show that the raspberry bud has a relation to air temperature similar to that possessed by the apple (Fig. 8). Both are warmed more than the air during sunshine, and are cooled below the air temperature at night, or when sunlight is diminished. The cooling is doubtless caused by evaporation, as was established for the apple (Grainger & Allen, 1936). Both apple and raspberry buds are occasionally cooled below the air temperature by a sudden burst of sunshine, acting through increase in evaporation, but the continuous effects of evaporation are not shown until the solar radiation diminishes (e.g. Fig. 8, II and III, 3–7 p.m.).

A simple experiment confirms the fact that water evaporates from raspberry buds. Twelve 6 in. pieces of dormant 1937 cane were separated into two equal groups. One group had the cut ends of the stem-pieces coated with vaseline, whilst the other group had all the buds coated in

addition. Each group was weighed, hung in the laboratory for seven days, and then reweighed. The group with untreated buds lost 19% of its original weight, whilst the vaseline coat upon buds of the other group reduced the loss in weight to 12%.
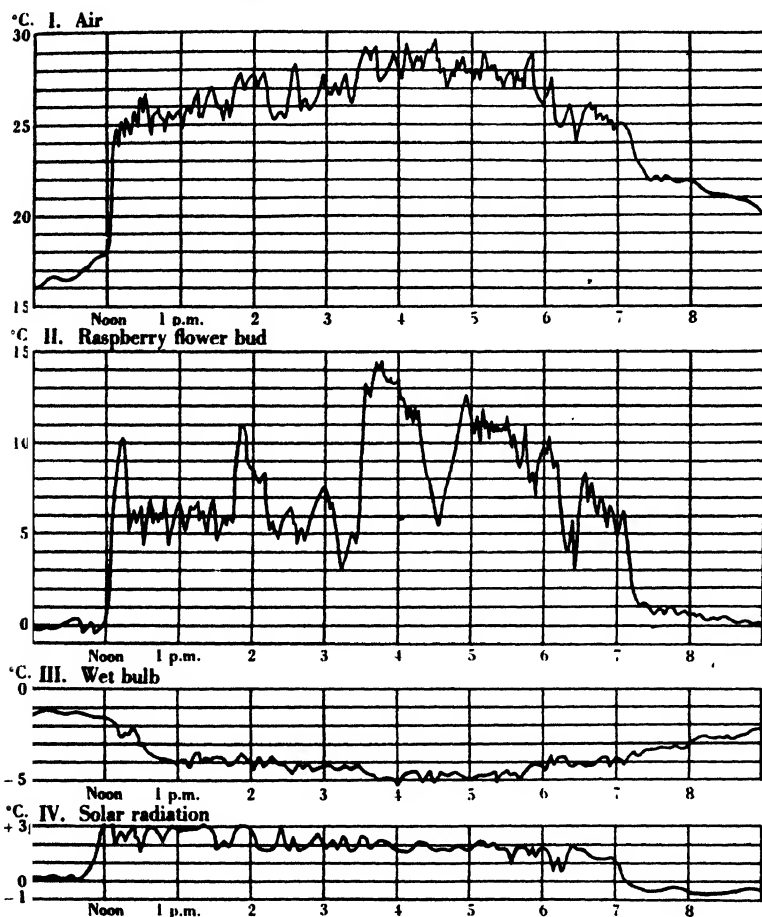
Fig. 9. I. Air temperature. II. Temperature of raspberry flower bud in relation to the surrounding air. III. Wet-bulb temperature. IV. Solar radiation. 20 June 1936.

Raspberry buds in the dormant state respond to rain as do apple buds (Grainger & Allen, 1936). There is a decrease in temperature, in relation to the air, whilst the rain is falling, but a quick recovery after it has ceased.

Raspberry flower buds, flowers, and developing fruits are all notable for the large amplitude of their temperature curves in sunshine. Fig. 9, which represents the temperature relations of an opening flower bud, is typical of all the stages mentioned above, and in all thirty-nine records have been taken. There is an obvious coincidence of the peaks of the bud-temperature curve with those of the air-temperature record, and
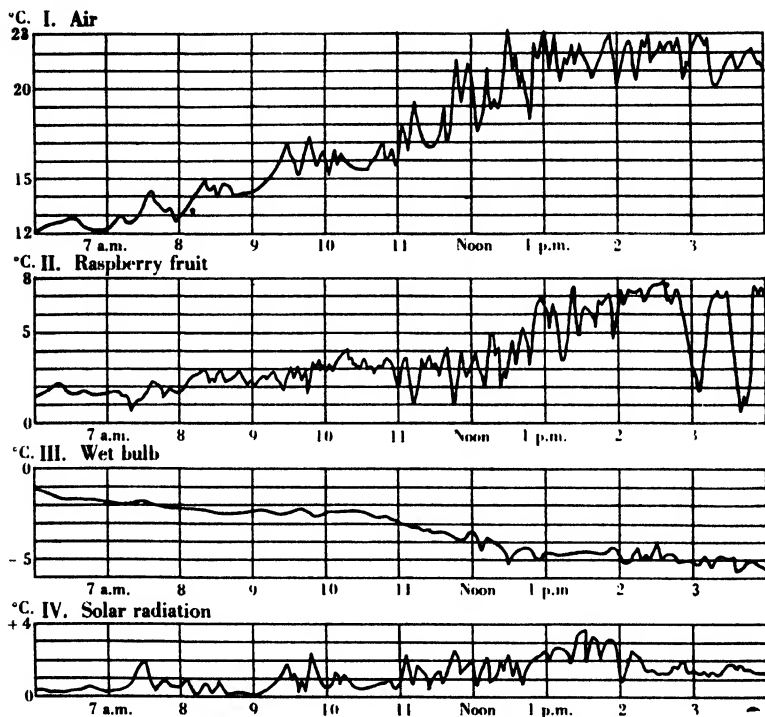
Fig. 10. I. Air temperature. II. Temperature of ripe raspberry fruit in relation to the surrounding air. III. Wet-bulb temperature. IV. Solar radiation. 16 July 1936.

also with the solar radiation maxima. Internal temperatures are often cooler than the surrounding air during the night. The rise in temperature of the ripe fruit during bright sunshine is less than with flower bud or flower (compare Figs. 9, 10), but is, however, greater than with the dormant bud. There are occasionally short periods, even during sunshine, when the fruit temperature descends below the temperature of the air, but the records are still very similar to those characteristic of the flower bud, flower and developing fruit.

The records of bud temperatures in the figures represent temperature *differences*. The curves must be added to those of the air temperatures for the same period, in order to represent the actual bud temperatures. The fact then emerges from Figs. 9 and 10 that the flower buds, flowers and fruits are often at a temperature of 40° C. (108° F.) during sunshine which only warms the air to 28° C. (81° F.). These figures are maxima, but they indicate the degree to which the small drupels can be warmed during sunshine, and probably help to explain the daily ripening of the raspberry crop which is so pronounced a feature of the soft-fruit industry. Table II shows that the raspberry has, moreover, the shortest period between the first flower and ripe fruit of any of the common species there listed. The information is compiled from averages for the years 1916–31 (Clark *et al.* 1933), and relates to Lowe's work in Worcestershire.

Table II. *Time in days between first flower and ripe*
fruit for several common trees and shrubs   ·

Alder, *Alnus glutinosa*	206
Apple, wild, *Pyrus malus*	157
Apple, cultivated, *Pyrus malus sativus*	126
Ash, common, *Fraxinus excelsior*	162
Ash, mountain, *Pyrus aucuparia*	90
Blackthorn, *Prunus spinosa*	156
Black currant, *Ribes nigrum*	73
Cherry, cultivated, *Cerasus vulgaris*	71
Elder, *Sambucus nigra*	91
Gooseberry, *Ribes grossularia*	95
Hawthorn, *Crataegus Oxyacantha*	110
Pear, *Pyrus communis*	157
Raspberry, *Rubus idaeus*	38

It is hoped to investigate further this suggested correlation between high internal temperature and quick ripening of the fruit.

DISCUSSION AND SUMMARY

1. The results here described follow a previous investigation (Grainger & Allen, 1936), where bud temperatures and air temperatures were recorded by thermo-electric methods. Humidity and solar radiation have also been recorded electrically in the present experiments.

2. Temperatures of apple buds show slight increases, in relation to air temperatures, during the early stages of a frost, particularly when the buds are bursting. This "frost compensation mechanism" is, however, very slight, and is of very little practical importance. Apple-bud temperatures during severe frosts in the dormant period usually show fairly close agreement with the air temperatures after the initial stages.

3. Experiments upon the control of frosts by the use of orchard

heaters burning crude oil show that their beneficial effects are due to warming of the air by convection, and not to heating of the bud by direct radiation. No difference in the bud-temperature record could be observed when the bud was exposed to, or shielded from, direct radiation from the flames. The internal temperature of an apple bud was uniformly lower than the temperature of the surrounding air during a frost in the dormant period, when orchard heaters were alight. Air temperatures round the bud were raised 2–3° C. above the surrounding air by the heaters.

Heaters with open flames appear to provide satisfactory control of frosts. The results here reported, however, raise the question as to whether a more efficient and economical type could not be evolved. Considerable heat must be lost by radiation from the flames to an open sky; lateral distribution of heat between heaters is not very good; and temperatures inside the bud are lowered by evaporation, owing to the drying effects of the heaters. It is suggested that a heater which would provide moist, heavy smoke would be a more efficient control of frosts than a flaming heater. Losses by radiation would be minimized, for smoke would have the additional advantage of providing insulation for the frost itself. Ground frosts or radiation frosts are caused by the radiation of heat from the earth to the heavens, and a blanket of smoke near the ground would diminish this loss. The inclusion of a small quantity of steam in the smoke would help to prevent the slight lowering of the bud's internal temperature by evaporation. Such a heating effect is provided by the practice of "smudging", where fires of partly dry refuse are used to provide moist smoke. The labour involved in building such fires is great, and an apparatus which would be as simple and cheap as the open flame oil heater, and yet would give a mixture of smoke and steam, should prove more efficient in practice. The heaters should also be disposed that any katabatic winds (Cornford, 1937) direct the heated air towards the trees. It would be somewhat easier to do this if lines of trees were planted diagonally across a slope, rather than straight up and down.

4. Two apple flower buds upon the same branch, and separated by only 4 in., may have divergent internal temperature relations, though a majority of records shows considerable agreement.

5. Temperature relations of raspberry buds, flowers and fruit have been studied. Dormant buds have internal temperatures which are similar to those of apple buds. They are warmed more than the air by solar radiation and are usually cooler than the air during the night, owing to evaporation from the buds.

Flower buds, flowers and developing raspberry fruits are warmed above the temperature of the air during sunshine, and may attain a temperature of 40° C. (108° F.) when the air is only at a temperature of 28° C. (81° F.). Such high temperatures may possibly explain the speedy ripening of the raspberry.

6. Estimations of the depression of freezing-points of standardized extracts from dormant and from bursting buds of hazel suggest that the sap cannot freeze more easily when the buds begin to open. They had, in fact, a greater depression of freezing-point at this time.

REFERENCES

CLARK, J. E., MARGARY, I. D. & CAVE, C. J. P. (1933). Report on the phenological observations in the British Isles from December 1931 to November 1932. *Quart. J.R. met. Soc.* **59**, No. 251.

CORNFORD, C. E. (1937). A note on frost damage investigations. *Ann. Rep. E. Malling Res. Sta.* 1936, pp. 126–30.

GRAINGER, J. & ALLEN, A. L. (1936). The internal temperatures of fruit-tree buds. *Ann. appl. Biol.* **23**, 1–10.

THE MANURIAL REQUIREMENTS OF PYRETHRUM (*CHRYSANTHEMUM CINERARIAEFOLIUM* TREV.)

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(With Plates I and II and 1 Text-figure)

INTRODUCTION

IT is generally recognized that the insecticidal pyrethrum plant (*Chrysanthemum cinerariaefolium* Trev.) prefers a comparatively dry climate and a well-drained sandy soil, and that its manurial requirements are small.

A valuable account of pyrethrum cultivation in Japan is given in the *Bulletin of the Imperial Institute* (1937).. Land exposed to the sun with sandy soil and good drainage is chosen, and stable litter with an auxiliary in the form of night soil, plant ash, fish cake or superphosphate is applied in moderate dressings. In fertile soil, the excessive application of nitrogenous manures results in leaf development at the expense of flower production. The stable litter is used to prepare the land for the seedlings, and the auxiliary manure is applied in the autumn after the flower harvest. Application of the auxiliary manure in the spring results in a reduced yield of flowers.

Gnadinger *et al.* (1936) found that the application of commercial fertilizers had little effect upon the yield of flowers or their content of the pyrethrins. Drain (1936), describing the cultivation of pyrethrum in Tennessee, states that the best results were obtained on fertile sloping soil, and that a moderate application of a complete fertilizer is desirable. Excessive application of nitrogen tended to reduce the yield of flowers, and predisposed the plants to disease. Ripert (1935) recommends the use of a complete fertilizer, stating that in favourable years considerable increases in flower production result, while in unfavourable years a satisfactory yield is maintained. Fertilizers with rapid action are recommended for dry years, and slow-acting fertilizers for wet years. Nitrate fertilizers are not to be recommended. Ripert states, also, that the use of a complete fertilizer has an effect upon the longevity of the crop.

In previous work (Martin & Tattersfield, 1934) experiments were carried out in large pots, using the heavy soil from Broadbalk field,

Rothamsted, which had not been manured for many years and, for each trial, rooted shoots from one parent plant. Weighed quantities of fertilizers were applied at the beginning of each experiment. The results showed that after a slight initial increase in the yield of flowers, the fertilizers had little effect upon the heads produced or upon their content of pyrethrin I. Good yields of flowers rich in pyrethrin I were obtained from the plants grown in the unmanured soil. The pyrethrin I content was found to be more dependent upon a genetical than a nutritional factor. It was hoped to obtain information upon the effect of the manures upon the survival period of the plants, but root development became so excessive as to necessitate the abandonment of the experiments.

The present paper deals mainly with a small-scale field experiment, designed to provide further information on the influence of manures upon the yield of flowers, their content of the pyrethrins, and upon the economic survival period of the plant. The aim of the experiment was discussed and its design decided upon by a committee composed of Mr C. T. Gimingham, Dr D. J. Watson and the authors, the manurial system being finally worked out by Dr D. J. Watson.

Experimental

The experiment was commenced early in 1933 on a hill in Roadpiece field, Woburn, Beds. The experimental area had previously been under grass with lucerne. The soil was very sandy, of the Lower Greensand type, with, in many parts, the characteristic ironstone pieces. The soil overlying the sand was very thin, and in order to bring the area into a low state of fertility and to eliminate any effect due to the previous crop, the surface soil was removed in March 1933 to a depth of 2–3 in. The area was enclosed in pig- and cattle-proof fencing, with wire netting to keep out ground game.

The experimental area was made up of thirty-two plots, each 6½ by 5 yd. or approximately 0·0067 acre in size. Guard rows separated the experimental plants, so that the area of each plot harvested was 0·0056 acre. There were 108 experimental plants in each plot, made up of nine rows of twelve plants each; the rows of plants and the plants in the rows were each 18 in. apart.

The plants used for the experiment were raised from seed at the Plant Pathological Laboratory of the Ministry of Agriculture, and were planted out at the end of May 1933. The lime had been applied to the appropriate plots at the end of April, and the mineral manures were applied immediately before planting. The nitrogenous manures were applied in two dressings, early in June and again in August. The climatic conditions were unsatisfactory for the planting out, and gaps were filled with new seedlings on two occasions in June. By August 1933, the plants were sturdy and were bushing out well, with a few gaps. These were filled in November with plants from the guard rows, and the latter were replanted with new seedlings in May 1934.

Manurial treatments. Two replications of combinations of no lime (O) and lime (L), no fish manure (O) and fish manure (F), no artificials (O) and complete artificials (A),

applied in the first year of the experiment only (1), and applied every year (2), were tested. Lime was applied in the first year of the experiment only (1933) at the rate of 2·88 tons of ground lime, equivalent to 4 tons of calcium carbonate/acre. Where applied in the first year only, fish manure was given at the rate of 5 cwt./acre, equivalent to 0·4 cwt. nitrogen/acre, and where applied every year it was given at half this rate per annum. The complete artificials were applied as sulphate of ammonia, super-phosphate and muriate of potash. Where applied in the first year only, they were given at rates equivalent to 0·4 cwt. nitrogen, 0·4 cwt. P_2O_5 and 0·5 cwt. K_2O/acre, and where applied every year half these rates were given per annum. The manures were applied in the spring of each year, those for 1934 being applied in April. The plan of the experiment is given in Text-fig. 1.

1							8
LOA 1	LFO 2	OFO 2	LOO 1	OOA 1	LOO 1	OOA 2	OOO 2
LFO 1	OOA 2	OOA 1	OFA 2	OFO 1	LOA 2	LOA 1	LFA 1
LFA 2	OFO 1	LFA 1	LOA 2	LFO 1	LOO 2	LFO 2	OFA 2
OOO 1	LOO 2	OOO 2	OFA 1	OFA 1	LFA 2	OOO 1	OFO 2
25							32

N.W.
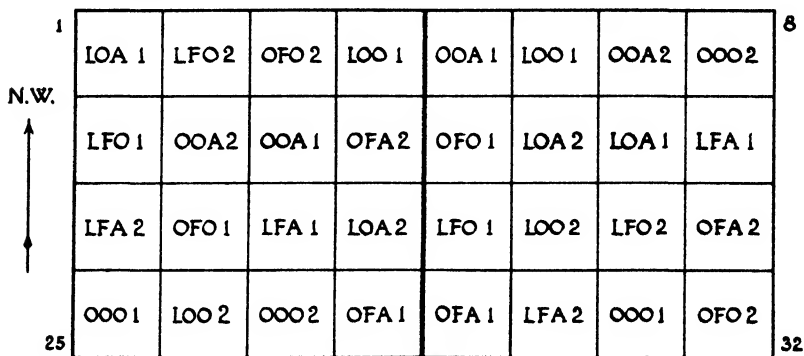
O = No application   1 · Manures applied first year only
L · Lime   2 · Manures applied every year
F · Fish Manure
A · Artificials   Lime applied in first year only

Text-fig. 1. Plan of the experimental area.

1934 *harvest*

The crop flowered well in June 1934, and was harvested 4–6 July, when the majority of the flowers were in the fully open condition. Owing to damage to the outside guard rows on the north-west and south-east sides of the experimental area, it was decided to abandon one row at each of these extremities. Eight rows of experimental plants were therefore harvested in plots 1–8 and 25–32. The flowers from each plot were harvested separately. It was found to be most convenient for the flowering stalks of each plant to be loosely tied, the bunches cut by shears, and the heads removed by drawing small bunches through metal combs (see Jary, 1936). The flowers, shielded from the sun by hessian cloths, were dried by exposure in an unheated glasshouse. The stalks were completely

removed, the yield from each plot determined, and representative samples of the air-dried flowers taken for analysis. The pyrethrins I and II were determined by the Tattersfield "acid" method as modified by Martin & Tattersfield (1931). The free acids were not removed and the dicarboxylic acid of the pyrethrin II was extracted with ether in a separating funnel. The yields of air-dried stalkless heads per plot, together with their pyrethrin contents, are given fully in the *Report of the Rothamsted Experimental Station for* 1934 (p. 203). The summarized results of the statistical examination are given later in this paper (Tables I–III).

At the time of the 1934 harvest 8·0% of the experimental plants had failed, and the gaps were filled from the guard rows in November 1934. The area was weeded in October and December 1934, and the manures were again applied, where required, in March 1935.

1935 *harvest*

The plants began to flower vigorously at the end of May, and all nine rows of each plot were harvested 8–12 June. Photographs were taken immediately before harvest (Pl. I, figs. 1, 2). The heavy crop necessitated five people beheading at the combs for the greater part of the 4½ days required for the harvesting. The flowers from each plot were dried as before, the stalks fully removed from a representative sample, and the weight of air-dried stalkless heads per plot calculated. The representative samples were analysed for pyrethrins I and II by the method of Seil (1934). The yields of air-dried stalkless flowers per plot are given in the *Report of the Rothamsted Experimental Station for* 1935 (p. 201), but the pyrethrin contents are not included, as they were determined at a later date. The values obtained have, however, been incorporated for reference purposes in the *Report of the Rothamsted Experimental Station for* 1937. The summarized results, following statistical examination, are given in Tables I–III.

The extent of failure of the experimental plants at the time of the 1935 harvest amounted to 5·7%. Large differences were seen in the development of weeds on the plots, those receiving fish manure or fish manures and artificials every year being generally in a worse condition than the plots receiving manures in the first year only. The plots were cleaned in September 1935. The gaps were not filled and the manures were again applied, where necessary, in April 1936.

1936 *harvest*

The flowers were harvested 7–10 July. Owing to the failure of plants in the outside guard rows of plots 1–8 and 25–32, only eight rows in these plots were harvested as in 1934. The flowers from each plot were weighed in the fresh condition, and a representative sample taken, on which the yield of oven-dried (100° C.) stalkless flowers was determined. The yields of dry stalkless heads were then calculated for the plots. The pyrethrins I and II contents were determined on air-dried flowers by the method of Seil, with preliminary removal of the free acids from the petroleum-ether extracts. The yields of oven-dried stalkless flowers per plot, with the pyrethrin contents of the air-dried flowers, may be obtained by reference to the *Report of the Rothamsted Experimental Station for* 1936 (p. 231). The summarized results, after statistical examination, are included in Tables I–III.

Records were taken of the numbers of plants surviving at the time of the 1936 harvest. Of the experimental plants, 20·1 % had failed. The plants in plots 1–8 were smaller than the remainder, and in these plots 32·4 % of the plants had died. Of the rows of plots, the second row made up of plots 9–16 showed 19·9% of plants missing, while plots 17–24 showed 14·0%, and plots 25–32 14·1% of failures. The progressive decrease in the percentage death of plants from plots 1–8 to plots 17–24 was probably influenced largely by the fact that the experimental area was not on level ground. Plots 1–8 were on the crest of the hill facing the north-west, and had experienced the full force of the winds from the north, while plots 9–16 and 17–24 were increasingly sheltered by the brow of the hill and by the plants in plots 1–8.

The experimental area was hand-hoed in August 1936, and again completely cleaned prior to the application of the manures in April 1937. It was decided at this stage to omit plots 1–8 and 25–32 when taking the 1937 harvest owing to the failure of the plants in plots 1–8 and the loss of the guard row in plots 25–32. The plots receiving manures in the first year only were to be regarded in the subsequent statistical analysis as unmanured plots, there being little chance of any residual manurial effect.

1937 *harvest*

The flowers from plots 9–24 were taken on 28 and 29 June. The fresh flowers from each plot were weighed, and the dry matter (100° C.) and stalk content determined on representative samples. The pyrethrins I and II were determined on air-dried stalkless flowers, containing approxi-

mately 12% of moisture, by the method of Seil, incorporating the preliminary removal of the free acids. The yields of oven-dried flowers for the plots harvested may be obtained from the *Report of the Rothamsted Experimental Station for* 1937, while the summarized results of the statistical examination of the data are included in Tables I–III.

Table I. *The effect of lime, applied in the first year only, upon the yield of flowers and their content of the pyrethrins*

Year	No lime	Lime	Difference	s.e. of diff.
		*Dry flowers (cwt./acre)		
1934	4·70	5·14	+0·44	±0·29
1935	6·72	7·32	+0·60	±0·37
1936	4·93	5·28	+0·35	±0·33
1937	4·26	4·86	+0·60	±0·45
		Pyrethrin I (% of flowers)		
1934	0·528	0·559	+0·031	±0·0201
1935	0·449	0·472	+0·023	±0·0207
1936	0·406	0·420	+0·014	±0·0231
1937	0·520	0·545	+0·025	±0·0183
		Total pyrethrins (% of flowers)		
1934	1·141	1·196	+0·055	—
1935	1·006	1·051	+0·045	—
1936	0·868	0·931	+0·063	—
1937	1·230	1·250	+0·020	—

Table II. *The effect of manures, applied in the first year only, upon the yield of flowers and their content of the pyrethrins*

Year	No manures	Artificials	Fish manure	Artificials plus fish manure	s.e.	Mean of manures
			*Dry flowers (cwt./acre)			
1934	4·94†	4·16	4·82	3·58	±0·404	4·19
1935	6·62†	7·00	7·75	7·51	±0·520	7·42
1936	5·27†	5·28	5·12	5·38	‡	5·26
1937	—	—	—	—	—	—
			Pyrethrin I (% of flowers)			
1934	0·55†	0·55	0·52	0·55	±0·0284	0·54
1935	0·46†	0·50	0·44	0·46	±0·0246	0·47
1936	0·38†	0·39	0·40	0·37	±0·0327	0·39
1937	—	—	—	—	—	—
			Total pyrethrins (% of flowers)			
1934	1·18	1·16	1·15	1·24	—	1·18
1935	1·01	1·11	1·00	1·03	—	1·05
1936	0·86	0·88	0·86	0·82	—	0·85
1937	—	—	—	—	—	—

* Yields for 1934–5 are on air-dry basis; for 1936–7 on oven-dry basis.

† The standard errors of these figures are $1/\sqrt{2}$ times the standard errors given for the corresponding manured plots.

‡ No single standard error is applicable to these figures.

Table III. *The effect of manures, half quantities applied every year, upon the yield of flowers and their content of the pyrethrins*

Year	No manures	Artificials	Fish manure	Artificials plus fish manure	S.E.	Mean of manures
			*Dry flowers (cwt./acre)			
1934	4·94†	5·28	6·03	5·60	±0·404	5·64
1935	6·62†	6·84	6·38	7·42	±0·520	6·88
1936	5·27†	5·00	4·76	4·78	‡	4·85
1937	4·17†	4·58	5·25	5·49	‡	5·11
			Pyrethrin I (% of flowers)			
1934	0·55†	0·54	0·54	0·54	±0·0284	0·54
1935	0·46†	0·45	0·45	0·46	±0·0246	0·45
1936	0·38†	0·47	0·46	0·45	±0·0327	0·46
1937	0·51†	0·57	0·55	0·55	‡	0·56
			Total pyrethrins (% of flowers)			
1934	1·18	1·14	1·14	1·16	—	1·15
1935	1·01	1·02	1·04	1·02	—	1·03
1936	0·86	1·00	0·95	0·97	—	0·97
1937	1·22	1·27	1·24	1·27	—	1·25

* Yields for 1934–5 are on air-dry basis; for 1936–7 on oven-dry basis.

† The standard errors of these figures are $1/\sqrt{2}$ times the standard errors given for the corresponding manured plots.

‡ No single standard error is applicable to these figures.

In the plots harvested, 28·1 % of the plants had failed, there being a greater loss in plots 9–16 (32·8 %) than in plots 17–24 (23·5 %). After the 1937 harvest the roots of one of the experimental plants were excavated to determine the extent of root development. There was comparatively little lateral spread, while from the mass of root material clustered near the surface, intertwined fine roots penetrated the sand to a depth of 5 ft. (Pl. II).

Weed population

The experimental area was not cultivated after August 1937, and observations on the prevalence of weeds were made in April 1938. Of the annual weeds, no chickweed, spurry or mayweed, and very little *Veronica*, were found, although these were abundant in the surrounding fields. *Poa annua* was abundant, and groundsel, *Alchemilla* and small leguminous weeds, probably wild clovers, were present. There had been a considerable amount of *Polygonum aviculare* in 1937, but no new seedlings were visible. There were many more perennial weeds, and these, particularly the grasses, were very abundant. Grasses predominated, chiefly *Agrostis stolonifera* and *Festuca ovina*, both very common in the neighbourhood. There were also many thistles, and patches of *Convolvulus*. There were at this stage no apparent differences in weed development between the plots manured in various ways or due to the effect of lime.

Climatic conditions

The months of 1933 during which the seedlings were establishing themselves were abnormally sunny, warm and dry, the succeeding winter months being normal. 1934 was generally a year of average sunshine and temperature, with low rainfall. July was sunnier and warmer than average, while December was particularly warm and wet. 1935 showed in general normal sunshine and temperatures, July again being sunnier, warmer and drier than usual. April, September and particularly November were wet months. The year 1935 was characterized by severe frost conditions in May, a minimum grass temperature of 21·5° F. being recorded on 17 May. The year 1936, and the first six months of 1937 were periods of reduced sunshine, generally normal temperatures, and abnormally high rainfall.

DISCUSSION

The outstanding result has, perhaps, been the high yields of flowers obtained throughout the experiment, in view of the infertile nature of the soil used. The yield reached a maximum in 1935, two years after the seedlings were planted out, in spite of the severe frosts in May which almost completely checked the flowering of other experimental beds of pyrethrum in lower-lying localities. Lime, which was applied in the first year only, increased the yield of flowers slightly each year (Table I). The increases were not individually significant in any year, and their regularity may partly be due to the fact that the same plots persisted throughout the experiment. The effect of lime upon the pyrethrin I contents of the flowers was very small but positive. The results are in agreement with the view generally held that lime is beneficial to the production of flowers.

Following the liberal application of manures in the first year only, a significant depression in the yield of flowers resulted in the following year (1934), succeeded by an almost significant increase in 1935, and no effect in 1936 (see Table II). There was no effect upon the pyrethrin I or total pyrethrin contents of the flowers. The initial depression in the yield of flowers obtained in 1934 may have resulted from too great a vegetative development of the plants, at the expense of flower production. This effect has been noted before, when plants showing a large, bushy development have produced few flowers.

When the manures were applied in moderate dressings every year, a significant increase in the yield of flowers resulted in 1934 and again in 1937 (see Table III). In 1935 the application of manures had no effect,

and the apparent depression in 1936 did not approach significance. In the two years in which the manures increased the yield of flowers, fish manure gave somewhat higher yields than did the artificials, though the differences were not significant. Manures applied every year had no effect upon the pyrethrin I content of the flowers in 1934 and 1935, but produced significant increases in 1936 and 1937. We have grown pyrethrum experimentally at various centres in England, and have noticed that, in general, the maximum yield of flowers is obtained in the second year after planting out, followed by a gradual drop in the total pyrethrin content of the flowers. The yearly application of moderate dressings of manures in this experiment has had the apparent effect of delaying this fall in pyrethrin content in the fourth and fifth years of the experiment. In all cases, the effects on the total pyrethrins were similar to those on the pyrethrin I content of the flowers.

The pyrethrin contents of the flowers in 1936 were generally lower ·than in the previous years, particularly on the unmanured plots, and this fact may have been connected with the adverse climatic conditions prevailing during the first half of the year. March, April, May and June were overcast months, while June was particularly wet. The first six months of 1937, however, were all dull and wet, and yet the pyrethrin contents of the flowers were amongst the highest recorded during the experiment. The high values for the 1937 flowers may have been influenced by the low pyrethrin contents of the flowers of the previous year.

There was no evidence in the fifth year of the experiment that the yearly application of moderate dressings of manures had played any part in prolonging the survival period of the crop, judged from the percentages of plants surviving. The plants grown in the plots to which no manures had been supplied were vigorous in the fifth year of the experiment, showed no greater percentage of failure than the plants grown in the manured plots, and flowered at the rate of 4 cwt. of dry flowers/acre. There was, however, some indication that lime produced a slight increase in plant survival in the last two years, as the following figures for the percentages of plant failure show:

	1934	1935	1936	1937
No lime	8·1	5·5	23·3	31·2
Lime	7·9	5·7	16·9	25·1

The manurial requirements of pyrethrum would appear to be surprisingly small, but the possible beneficial effect of moderate yearly dressings of manures upon the yield of flowers and upon the pyrethrin

content is seen, particularly in the last year of the experiment. The application of the manures in the autumn after flowering may prove advantageous, but was not tested in the present experiment as it was felt that manures so applied would rapidly be lost from the sandy soil by winter rain. The use of dung and organic manures may also have a valuable effect in improving the tilth of the soil. Further experimentation is required and should take place in the localities in which pyrethrum is grown commercially.

A note by Mr W. G. Cochran upon the statistical examination of the data is appended.

Statistical note. The experiment consisted of two randomized blocks of 16 treatments each and the statistical analysis followed the usual method for randomized blocks. In 1937 only half of the experiment was harvested and as this did not coincide with a block, an analysis was performed by the method of least squares, ignoring the difference between blocks. The standard errors per cent per plot may be of interest to those contemplating experiments on this crop. For dry flowers, they were 16·4% in 1934, 14·8% in 1935, 13·0% in 1936 and 17·8% in 1937. These figures are considerably higher than the usual average of 8–12% for English farm crops. It should be noted, however, that the plot size was only 0·0056 acre and that the positions of the blocks turned out to be unfortunate. Had the comparative failure of the outside rows (north-west and south-east) been foreseen at the start, a design in four randomized blocks of eight plots would have been more accurate. Further, owing to the factorial design used, the replication on the effect of lime was sixteenfold.

The pyrethrin figures in Table I will be found to differ slightly from the corresponding figures in the Rothamsted Experimental Station Report owing to the use of an extra decimal place in the calculations in this paper.

SUMMARY

A small field experiment upon the manurial requirements of the insecticidal pyrethrum plant, grown upon sandy soil of low fertility, is described. Lime had the effect of producing slight, but not significant, increases each year in the yield of flowers and their content of the pyrethrins, and decreased the percentages of plant failures in the fourth and fifth years of the experiment. There was a significant depression in the yield of flowers in the year after the single application of double dressings of the manures, but no effect in later years. The yearly application of moderate dressings of manures gave significant increases in the

yield of flowers in the second and fifth years, and significant increases in the pyrethrin I content of the flowers in the fourth and fifth years of the experiment.

We wish to convey our thanks to Mr C. T. Gimingham for raising the seedlings used in the experiment and for helpful criticism and advice, to Dr D. J. Watson for assistance in drawing up the plan of the experiment, to the staff of the Woburn Experimental Station for assistance in the field work, and to Mr W. G. Cochran for carrying out the statistical examinations. The photographs were taken by Mr V. Stansfield.

REFERENCES

Anon. (1937). The cultivation of pyrethrum in Japan. *Bull. Imp. Inst., Lond.,* **35,** 318.

DRAIN, B. D. (1936). Pyrethrum in Tennessee. *Circ. Tenn. agric. Exp. Sta.* no. 59.

GNADINGER, C. B., EVANS, L. E. & CORL, C. S. (1936). Pyrethrum plant investigations in Colorado. *Bull. Colo. agric. Exp. Sta.* 428.

JARY, S. G. (1936). Pyrethrum. *J. S.-E. agric. Coll. Wye,* no. 38, p. 59.

MARTIN, J. T. & TATTERSFIELD, F. (1931). The evaluation of pyrethrum flowers *(C. cinerariaefolium). J. agric. Sci.* **21,** 115.

—— —— (1934). The effect of environmental conditions upon pyrethrum *(C. cinerariaefolium).* I. *Ann. appl. Biol.* **21,** 670.

Rep. Rothamst. exp. Sta. (1934–7).

RIPERT, J. (1935). *Le pyrèthre français.* Paris: Dehon and Co.

SEIL, H. A. (1934). Estimation of pyrethrins. *Soap,* **10,** No. 5, 89.

EXPLANATION OF PLATES I AND II

PLATE I

Fig. 1. The experimental plot immediately before the 1935 harvest.

Fig. 2. Near view of the experimental plot immediately before the 1935 harvest.

PLATE II

The excavated root system of a single plant of *Chrysanthemum cinerariaefolium.*

Fig. 1.

Fig. 2.

MARTIN, MANN and TATTERSFIELD.—The Manurial Requirements of Pyrethrum
(*Chrysanthemum cinerariaefolium* Trev.) (pp. 14–24)
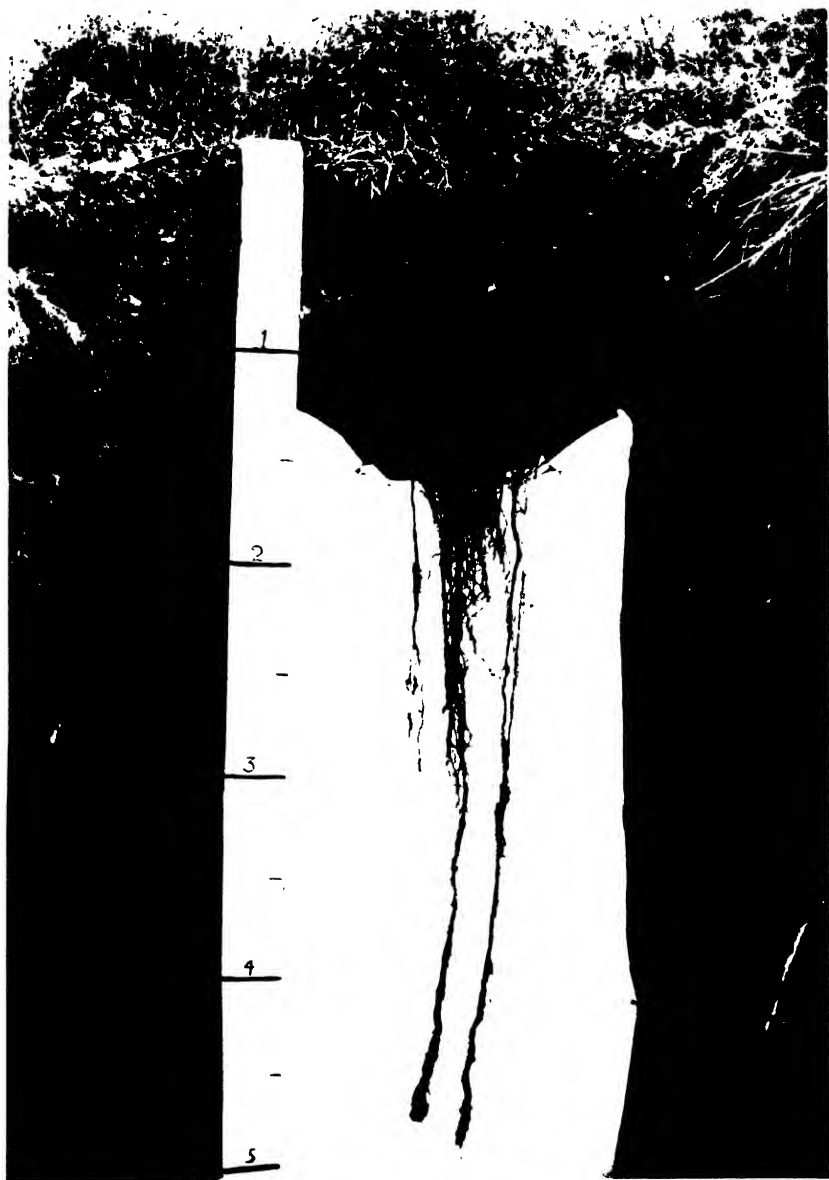
MARTIN, MANN AND TATTERSFIELD.—THE MANURIAL REQUIREMENTS OF PYRETHRUM
(CHRYSANTHEMUM CINERARIAEFOLIUM TREV.) (pp. 14–24)

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SOIL CONDITIONS AND THE TAKE-ALL DISEASE OF WHEAT

IV. FACTORS LIMITING INFECTION BY ASCOSPORES OF *OPHIOBOLUS GRAMINIS*

By S. D. GARRETT

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(With Plate III)

CONTENTS

I. INTRODUCTION

AFTER finding that the ascospores of *Ophiobolus graminis* are ejected from their perithecia into the air, Samuel & Garrett (1933) suggested that aerial dispersal of ascospores in showery weather might be responsible for the widespread epidemics of the take-all disease that occurred in South Australia in seasons of good spring rainfall. It was considered that the late appearance of the disease in the whiteheads form over whole fields of wheat might be due to infection from ascospores produced on plants killed early. The ascospore dispersal hypothesis thus seemed to account not only for the sudden appearance of whiteheads at heading time in crops that had shown little sign of the disease earlier, but also for the apparent limitation of widespread outbreaks of the disease to years of good spring rainfall in South Australia (Garrett, 1934).

Subsequent work (Garrett, 1936) on the subterranean activity of *O. graminis* on the wheat root-system has rendered an alternative explanation increasingly probable, though not necessarily to the exclusion of the ascospore dispersal hypothesis. This later work has shown that *Ophiobolus* spreads underground along the wheat roots at a rate which varies greatly with the nature and condition of the soil. If the amount of infectious material surviving in the soil from a previous diseased crop be

sufficient, a severe attack of the disease may occur on any soil and in any season. But with a sparser distribution of infectious material in the soil, the production of whiteheads will follow only on such soils and in such seasons as are favourable to the subterranean spread of the fungus. Thus, these general outbreaks of whiteheads occurred in South Australia only on the light-textured alkaline soils of the "mallee" areas, and only in years of good spring rainfall. Such soils, when the moisture content is adequate, have been shown to be very favourable to the spread of *Ophiobolus* along the roots (Garrett, 1934); in dry seasons, however, the drying-out of the surface soil may be expected to check the mycelial advance of the fungus towards the crown of the plant, and hence to retard or prevent altogether the production of whiteheads. Soil moisture is more likely to act as a factor limiting mycelial advance in South Australia than in England, where soil moisture content is generally higher throughout the growing season.

Gradual subterranean spread of the fungus from numerous scattered foci of infection can probably thus account, equally well with the hypothesis of ascospore dispersal, for these two features of take-all outbreaks in South Australia, viz. the unexpected development of whiteheads in full-sized plants with the advent of the first hot ripening weather, and limitation to seasons of good spring rainfall. An adequate investigation of ascospore infection is desirable in order that the relative importance of soil infection and ascospore infection may be assessed, and control measures directed accordingly. This need has recently been emphasized by Samuel (1937), in discussing the field occurrence of the disease in England.

II. EXPERIMENTAL

Perithecia of *O. graminis* were obtained in abundance by the following method. Wheat seedlings were planted above agar inoculum of the fungus in 2.8×20 cm. boiling tubes, filled to a height of 8 cm. with sand moistened with a standard nutrient solution for wheat plants. The sand was brought to a moisture content of 50 % saturation by the addition of this nutrient solution (75 g. dry sand and 12 c.c. solution per tube). Five wheat seeds were planted over an agar inoculum disk of *Ophiobolus*, and covered with moist sand. The tubes were plugged with cotton-wool and incubated in glass jars kept at laboratory temperature (16–20° C.) in a north window. Perithecia usually began to form on the infected roots and stems exposed to the light after a period of 2–3 weeks; mature ascospores could generally be obtained from such perithecia after 6 weeks.

The success of this method may be attributed to the provision of the natural substrate, an atmosphere of sufficient humidity, an adequate light intensity, and to the fact that, deliberately, other micro-organisms were not excluded. The stimulating effect of other micro-organisms upon the production of the perfect stage of fungi growing upon agar may frequently be observed, and has been investigated critically by Asthana & Hawker (1936), Hawker (1936) and others. From the ripe perithecia produced in these tubes, a suspension of ascospores could be obtained by soaking the perithecia-bearing stems and roots in water, and then straining the resulting suspension of spores through muslin. Smaller numbers of ascospores in bacteriologically sterile suspension could be obtained conveniently by allowing moistened ripe perithecia to eject their ascospores on to sterile coverslips $\frac{1}{2}$-1 mm. above the necks, as already described by Samuel & Garrett (1933).

No difficulty in obtaining ascospore infection had been anticipated, since mature ascospores always germinated readily on nutrient agars, and infection could always be obtained with the resulting agar cultures. Many of the isolates employed in preceding work with the fungus were derived originally from ascospores. It was, therefore, surprising to find that a series of infection experiments with ascospores gave completely negative results. Ascospores obtained as described above, and employed as soon as mature, generally showed a germination of more than 90 % on 0·5 % dextrose agar, yet infection failed to occur on a variety of soil types, either with germinating seeds or with seedlings of different ages. No infection was obtained, again, in pure sand, or in sand with an admixture of 1/3 or 1/4 of a steamed alkaline soil, known to be especially favourable to the spread of the fungus along the roots. The majority of infection experiments were conducted with a suspension of some 30,000 ascospores per c.c., of which 1 c.c. was added to the soil around each seedling, or to each planting hole for germinating seeds. In one experiment, 1 c.c. of a suspension containing 80,000 ascospores per c.c. was used per planting hole, but with equal lack of success. Infection of the roots of wheat seedlings by *Ophiobolus* is normally revealed by discoloration of the tissues within one week (at 20° C.) of inoculation. Roots of the ascospore-inoculated seedlings were searched for lesions and for the presence of runner hyphae of *Ophiobolus* under the binocular dissecting microscope, at a period of 2–3 weeks after inoculation, but without success. Ascospore-inoculated plants were also grown for a period of 2 months in the glasshouse, but the longer period of incubation again failed to reveal any trace of infection.

Since ascospores of *Ophiobolus* used in these experiments showed a

germination exceeding 90 % on 0·5 % dextrose agar, the mere increase of density in the spore suspension used for inoculation did not seem likely to lead to infection. Consideration of the fact that a nutrient agar culture derived from the ascospores would always give infection led to realization that the factor limiting ascospore infection might possibly be a nutritive one. Thus Brown (1922) found that spores of *Botrytis cinerea*, if sown in water on the healthy, uninjured leaf of broad bean, were unable to cause infection; if they were sown in nutrient solution (turnip extract), infection occurred at once. Brown further demonstrated a relationship between exosmosis of solutes from uninjured flower petals of different species, as measured by the conductivity of the infection drop, and their susceptibility to infection by the spores of *Botrytis*. It appeared possible that the ascospores of *Ophiobolus* might be able to cause infection of the seedling roots if a suitable extraneous nutrient were provided. One-half per cent of glucose was therefore added to the ascospore suspension in several of the infection experiments, but infection still failed to occur.

The analogy between spore infection by a leaf-infecting fungus such as *Botrytis* and a root-infecting fungus such as *Ophiobolus* is incomplete, however, inasmuch as spore germination and root infection by *Ophiobolus* are likely to be greatly impeded, if not inhibited, by the competition and antagonism of saprophytes developing on the glucose or other nutrient material added. For the production of conditions as favourable to infection as those obtaining in the *Botrytis* infection drop on the leaf, it would be necessary not only to present the ascospores of *Ophiobolus* with an accessary nutrient, but also to protect this nutrient from the rapid development of other soil micro-organisms. Sterilized soil is an environment satisfying both these requirements, for it serves as a suitable culture medium for *Ophiobolus* without the addition of further nutrients than those already set free by the process of heat sterilization.

An experiment was, therefore, set up to test sterilized soil as an infection medium for the ascospores of *Ophiobolus*. The soil employed was from Bridgham, Norfolk—a very light sandy loam of the Breckland type, overlying chalk and well supplied with calcium carbonate (*p*H value of soil approx. 8·0). This soil had previously been found very favourable to spread of the fungus along the roots, on account of its physical and chemical properties (Garrett, 1936). The experiment was conducted in large test tubes, 2·0 × 17·5 cm., which were filled to a depth of 6·5 cm. by the addition of 28 g. air-dry Bridgham soil; 5 c.c. of distilled water was then added to each tube to bring the soil to a moisture content of approximately 50 % saturation. Two dozen tubes were thus filled with soil,

plugged with cotton-wool, and autoclaved for 1 hr. at 1½ atm. A sterile ascospore suspension was obtained by allowing moistened ripe perithecia to eject on to a sterile coverslip. One platinum wire loopful of the sterile suspension was added to each tube of sterilized soil. A sterilized, pre-soaked wheat grain was then dropped into each tube, and covered with moist sterile sand. Four of the twenty-four tubes were, however, deliberately recontaminated by addition of a trace of unsterilized soil to the ascospore suspension. For a comparison in unsterilized soil, twelve similar tubes, and twelve larger tubes, of size 2·8 × 20 cm. (to permit of longer growth of the wheat seedlings), were filled with the same weight, 28 g., of air-dry Bridgham soil. The soil of each tube was brought to a moisture content of 50 % saturation by the addition of 5 c.c. of an ascospore suspension containing 24,000 ascospores per c.c., making 120,000 ascospores per tube. Germination of the ascospores on 0·5 % dextrose agar was estimated at over 90 %. Two wheat seeds were planted per tube and covered with moist sand. All tubes were kept in a north window at laboratory temperature, which fluctuated between 15 and 20° C. during the period of the experiment.

After one month, the wheat seedlings were dead in seventeen of the twenty tubes of sterilized soil inoculated with a sterile ascospore suspension and kept sterile. Examination of the roots showed severe infection and stunting by *Ophiobolus*, which had established itself in pure culture on the sterilized soil in these seventeen tubes. In the three tubes in which the seedlings remained alive, the roots of one seedling showed infection, but those of the other two remained healthy. Microscopical examination of all tubes before washing out had previously shown that whereas *Ophiobolus* was growing in pure culture throughout the soil of the seventeen tubes with dead seedlings, it had not succeeded in establishing itself throughout the soil of the other three tubes. This failure was attributed to chance contamination of the sterilized soil by other micro-organisms; in accordance with expectation, the seedlings in the four tubes deliberately recontaminated were found to be alive and healthy. In the twenty-four tubes of unsterilized soil, each inoculated with some 120,000 ascospores, all wheat seedlings were alive and apparently healthy; seedlings were washed out of the twelve smaller tubes at the end of the first month, but those in the larger tubes were left for a period of two months before root examination was made. Microscopical examination failed to reveal root infection in any of the forty-eight plants from the unsterilized soil.

This experiment was repeated, with essentially similar results. It then became necessary to determine whether ascospore infection could

take place in a sterile sand medium, with no other nutrients present than those deriving from the roots of the wheat seedlings. Since sterile sand offers no substrate for the multiplication of *Ophiobolus* mycelium, a large quantity of ascospores in bacteriologically sterile suspension was required for this experiment; it was obtained from perithecia which had formed in some cultures of *Ophiobolus* on oatmeal agar. The experiment comprised eighteen large test tubes (2·0 × 17·5 cm.), six with sterilized sand plus 1 % glucose solution, six with sterilized sand without nutrient, and six with unsterilized sand without nutrient. Two sterilized and presoaked wheat seeds were planted per tube, and each tube received 1 c.c. of a bacteriologically sterile ascospore suspension containing some 14,000 ascospores. The two series of tubes with sterilized sand were planted and inoculated with full aseptic precautions. All tubes were then incubated in a glass jar kept in a north window of the laboratory (at 16–20° C.). After 14 days, severe root infection in both the series of sterile sand could be seen through the glass walls of the tubes; seedlings were washed out after 19 days. In both series of plants grown in sterile sand, the roots were discoloured brown throughout, and had been killed off short by the infection; stem lesions were also present on every plant. No infection by *Ophiobolus* could be found in the roots of the plants grown in the unsterilized sand. The striking difference in appearance between the severely infected plants from the sterilized sand (without glucose) and the healthy plants from the unsterilized sand is shown in Pl. III. Infection was perhaps slightly more severe in the sterilized sand with glucose; the effect of glucose would probably have been more apparent had a lower concentration of ascospores been used for inoculation.

These experimental results are thus in agreement with those of Kirby (1925), who obtained infection by ascospores of sterile wheat seedlings growing in pure culture in tubes or Petri dishes on 0·2 % dextrose and potato agars. Mangin (1899) has figured infection of a wheat root hair by the germ tube of an ascospore. No other circumstantial accounts of ascospore infection have been found, though it seems to have been generally assumed that ascospore infection could occur, even if it were not of much importance in the dispersal or survival of the fungus.

III. Discussion

The occurrence of ascospore infection in sterile sand indicates that the nutrients provided by sterilized soil are unnecessary for the initiation of infection, and that sterile conditions alone are sufficient. It appears,

therefore, that the antagonism of other micro-organisms in some way prevents initiation of ascospore infection in unsterilized soils and even in unsterilized sand. Infection by agar inoculum disks of *Ophiobolus*, on the other hand, occurs freely in sand and in a variety of soils, and seems to be limited rather by physico-chemical conditions than by the antagonism of the soil microflora (Garrett, 1936). It is possible, however, that initial infection of a root by *Ophiobolus* can only occur at a certain nutritional level of the penetrating hyphae. The use of agar inoculum disks or of pieces of infected straw secures this nutritional level. In wheat seedlings grown under pure culture conditions in sterile sand, the excretion of organic material from the growing roots may provide the accessary nutrient necessary for successful ascospore infection. Work on root excretion and related phenomena has been reviewed recently by Loehwing (1937). In unsterilized soil and sand, however, these organic excretions and detrital material are rapidly assimilated by the general soil microflora. This aspect of the association of roots and soil micro-organisms has been investigated exhaustively by Starkey (1929–38), who has demonstrated a remarkable increase in numbers and activity of soil micro-organisms in the immediate vicinity of plant roots.

The observation that unsterilized soil and sand inhibits infection by ascospores but not by agar inoculum suggests that the inhibiting effect is connected in some way with the nutrition of the infection hyphae. Under sterile conditions, root excretions are wholly available to the ascospores and may make good the nutritional deficiency; in unsterilized soil, the root excretions are rapidly assimilated by the general soil microflora.

The relation of nutrition to germination and infection of the host by fungus spores has been reviewed recently by Brown (1936); it is becoming apparent that infection by some of the root-parasitic fungi is conditioned similarly by the nutritional level of the inoculum (Garrett, 1938*b*). In general, the greater the resistance offered by the host root to invasion, the higher must be the nutritional level of the fungus inoculum for successful penetration to occur. Thus, direct infection of the uninjured cork-covered older roots of forest and plantation trees by fungus spores has never been demonstrated and, in recent years, the importance of a "food base" behind the invading hyphae has been emphasized increasingly by Gadd (1936) and others.

In conclusion, the bearing of these results on the field occurrence of the take-all disease calls for some comment. It scarcely now seems likely that the ascospores can play any part in the propagation and dispersal

of the disease in the field, so that greater emphasis is laid on the underground survival and spread of the fungus, which is present probably to some extent in the majority of wheat-growing soils over its geographical range. The "sudden appearance" of the disease in a field where it has not been seen for some years is, therefore, to be attributed to changes in season or in farming practice, e.g. short rotations, which have favoured the fungus. This conclusion is supported by field observations made on the occurrence of the disease in the southern and eastern counties of England during the past few seasons (Garrett, 1938*a*). Severely diseased and clean crops were, not infrequently, seen standing side by side on the same land; such crops were sometimes on the two halves of one field on which different rotations had been followed. The extension of the disease to fresh areas may, therefore, be a slower process than has appeared hitherto; in order to explain it we seem to be thrown back once more on the original suggestion of McAlpine (1902) that dispersal is brought about by means of wind-blown fragments of infected plant material.

IV. SUMMARY

Attempts to produce infection of wheat seedlings by the ascospores of *Ophiobolus graminis* in a variety of natural soils and in sand have failed. Yet, the ascospores germinate well on nutrient agars, and the resulting agar cultures produce infection as readily as cultures obtained from mycelium.

Ascospore infection of sterile wheat seedlings growing in sterilized soil may be obtained without difficulty. The nutrients present in sterilized soil are unnecessary for the initiation of ascospore infection, which occurs as freely as in sterile sand. Ascospore infection is, therefore, considered to be inhibited in unsterilized soils and sand by the antagonism or competition of other soil micro-organisms.

In unsterilized sand this antagonism is not sufficient to hinder infection by agar inoculum, nor does it appreciably impede the progress of infection along the roots. It is suggested, therefore, that microbiological interference with the initiation of ascospore infection is a competitive rather than an antagonistic effect, and is due to assimilation by the general soil microflora of the nutritive substances excreted from the growing and developing roots. Only under sterile conditions is this organic detritus available to the germinating ascospores.

The results of these experiments render it unlikely that the ascospores can play any part in the survival and dispersal of the fungus under field conditions.

(a)

(b)

I am much indebted to Mr Geoffrey Samuel for criticism of the experiments.

REFERENCES

ASTHANA, R. P. & HAWKER, L. E. (1936). The influence of certain fungi on the sporulation of *Melanospora destruens* and of some other Ascomycetes. *Ann. Bot., Lond.*, **50**, 325.

BROWN, W. (1922). Studies in the physiology of parasitism. VIII. *Ann. Bot., Lond.*, **36**, 101.

—— (1936). The physiology of host-parasite relations. *Bot. Rev.* **2**, 236.

GADD, C. H. (1936). Diseases of the tea bush. II. Root diseases. *Tea Quart.* **9**, 5.

GARRETT, S. D. (1934). Factors affecting the severity of take-all. I, II and III. *J. Agric. S. Aust.* **37**, 664, 799 and 976.

—— (1936). Soil conditions and the take-all disease of wheat. *Ann. appl. Biol.* **23**, 667.

—— (1938a). The take-all or whiteheads disease of wheat and barley, and its control. *J.R. agric. Soc.* **98**, 24.

—— (1938b). Soil conditions and the root-infecting fungi. *Biol. Rev.* **13**, 159.

HAWKER, L. E. (1936). The effect of certain accessary growth substances on the sporulation of *Melanospora destruens* and of some other fungi. *Ann. Bot., Lond.*, **50**, 699.

KIRBY, R. S. (1925). The take-all disease of cereals and grasses caused by the fungus *Ophiobolus cariceti*. *Mem. Cornell agric. Exp. Sta.* no. 88.

LOEHWING, W. F. (1937). Root interactions of plants. *Bot. Rev.* **3**, 195.

MCALPINE, D. (1902). Take-all in wheat. *J. Dep. Agric. Vict.* **1**, 74.

MANGIN, L. (1899). Sur le piétin ou maladie du pied du blé. *Bull. Soc. mycol. Fr.* **15**, 210.

SAMUEL, G. (1937). Whiteheads or take-all in wheat. *J. Minist. Agric.* **44**, 231.

SAMUEL, G. & GARRETT, S. D. (1933). Ascospore discharge in *Ophiobolus graminis*, and its probable relation to the development of whiteheads in wheat. *Phytopathology*, **23**, 721.

STARKEY, R. L. (1929–38). Some influences of the development of higher plants upon the micro-organisms in the soil. *Soil Sci.* **27**, 319, 355 and 433; **32**, 367 and 395; **45**, 207.

EXPLANATION OF PLATE III

Wheat seedlings inoculated with ascospores of *Ophiobolus graminis*: (a) in unsterilized sand, (b) under sterile conditions in sterilized sand.

STUDIES ON *PUCCINIA ANOMALA* ROST.

I. PHYSIOLOGIC RACES ON CULTIVATED BARLEYS

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CONTENTS

INTRODUCTION

COMPARATIVELY little is known of physiologic specialization in *Puccinia anomala* Rost. The first account of experiments on the relative resistance of agronomic varieties of barley to *P. anomala* was published by Vavilov (1919). His results on eighty-two barleys showed the existence of resistant varieties, but it is not possible to compare his results with the reactions of existing physiologic races because there is no certainty that he was working with a pure inoculum from one physiologic race only.

Mains (1926) first drew attention to different physiologic races in *P. anomala* in the following statement: "Two physiologic forms of the leaf rust of barley have been distinguished by differences in reaction of the lines Oderbrucker C.I. no. 940, Featherston C.I. no. 1120 and Horsford C.I. no. 5057, all of which show high resistance to physiologic form 1 of *P. anomala* and are more or less susceptible to physiologic form 2."

Waterhouse (1927) published the reactions of 116 barleys inoculated with one stock culture of Australian rust. The barleys were from the species *Hordeum vulgare* L., *H. intermedium* Kake, *H. distichon* L., *H. deficiens* Steud., and also two wild barleys, *H. spontanenum* Kock and

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H. murinum L. He stated that only seventeen barleys were resistant to his strain of *P. anomala*, fifteen belonging to *Hordeum vulgare* L., one to *H. distichon* L., and the other was *H. murinum* L. All the remaining barleys were more or less susceptible. Waterhouse (1929), in a new account of his experiments, stated that some of the varieties, which were resistant during the winter, were more or less susceptible in summer. Also, he emphasized that, in his new experiments, *H. murinum* was susceptible to *P. anomala*. This is an important point since no one else has recorded infection on this barley, and hitherto it has been considered immune. It seems probable that the new culture of Waterhouse was a physiologic race not yet studied.

Mains (1930) summarized his work on this rust. He used cultivated and wild barleys and also several grasses and gave the reactions of some of his agronomic varieties of barley to his two physiologic races of *P. anomala*. Also, he tested these races on twenty-six of the most resistant of the barleys used by Waterhouse.

Hirchhorn (1933), in Argentina, on the basis of field observations, reached the conclusion that *P. anomala* in La Plata exists in physiologic races different from those of Mains and Waterhouse.

. Mains & Martini (1932) published a new account of the reactions of Mains's two physiologic races on about 600 varieties and selections of barleys in the greenhouse and in the field.

Brown (1931) described physiologic races of *P. anomala* found in Canada in 1929. These races were distinguished from one another on six new barleys, all different from Mains's differential hosts. Brown claimed that four physiologic races were differentiated in this way.

The most valuable selection of barleys was made by Hey (1932) from 273 barleys of the species *Hordeum polystichum* and *H. distichum*, from which he selected ten differential hosts. With these he was able to distinguish eight physiologic races in Germany and Bulgaria.

Again, in Germany, Ronsdorf (1934), working with Hey's barleys and with a new variety Aegyptische 4-zeilige (selected by Gassner & Straib, 1932), was able to identify two of Hey's races, numbers III and IV, and also a new race, which she numbered IX. Ronsdorf (1935) described two new races from America, X and XI, and also published the reactions of races II, III, IV, V and IX on some of Mains's differential barleys.

Stakman *et al.* (1935) referred to Hey's (1932) and Rondsdorf's (1934) results and modified the list of barleys selected by Hey. A new barley was added (Breuns Neuzucht 25) which had been tested but not selected by Hey. Also one of Hey's selected barleys was omitted.

<div align="center">MATERIAL AND METHODS</div>

<div align="center">(1) *Sources of cultures of* P. anomala *Rost.*</div>

Seventy-seven collections of *P. anomala* from cultivated barleys, obtained from England, Portugal and Spain, were tested between February 1933 and June 1936.

All the remaining cultures were obtained from aecidia produced in 1936.

<div align="center">(2) *Establishment of cultures*</div>

Whenever possible single-spore cultures were prepared from field material as soon as it was received. When the material arrived in bad condition or during a busy time a mass inoculation was made and single-spore cultures were established later.

(*a*) *Mass inoculations.*

A pot of eight to ten seedlings of Spratt Archer barley was used. Each plant in the pot was inoculated with spores taken from a single pustule chosen at random from the most isolated pustules on the leaf.

Incubation took place under bell-jars in which the atmosphere was kept damp. Sometimes during summer in Portugal it was necessary to line one-half of the bell-jars with damp blotting paper, and when the temperature rose above 26° C. the air was cooled with ice.

After 48 hr. incubation in the moist chamber the pots were transferred to benches in the greenhouse and the plants were kept under lamp glasses. The top of each lamp glass was covered with a layer of cotton-wool over which a small square of muslin was placed, the whole being held by an elastic band. If spore-proof cellophane cylinders were available these were used instead of the lamp glasses, since they allowed the air inside to be kept fairly dry.

(*b*) *Establishment of single-spore cultures.*

To establish single-spore cultures three methods were tried, the agar method, Newton & Johnson's method (1932), and also a new method consisting of transfer of the spores from a dry glass slide with a wet glass capillary needle. The capillary tube was dipped in sterile water and used to pick up individual spores from the slide. The spores were transferred to the leaf by blowing gently through the mouthpiece of the pipette. The pipettes were sterilized between inoculations. The barley seedlings were then kept at room temperature until the droplets of water evaporated; this was done to prevent the drops from running down the leaf and mixing the spores. Incubation was allowed for 48 hr. as described by Newton & Johnson (1932), after which the pots were covered with cellophane cylinders. With this method the average number of infections obtained was 20–50%. As soon as the flecks of pustules were visible only one was allowed to develop on each leaf. The remaining flecks were covered with a layer of vaseline on both sides of the leaf. Each leaf was then numbered and covered with a test-tube kept in position by a zinc support. Three days after the pustules had made their appearance, uredospores were taken from each pustule to a new pot of barley seedlings in order to establish stock cultures.

<div align="center">(3) *Maintenance and preservation of stock cultures*</div>

All stock cultures were maintained on seedlings of Spratt Archer barley kept under the cellophane spore-proof cages or in the rust-free room of the greenhouse. Inoculations were made on the first leaf of the seedlings when the second leaf began to appear.

The inoculum was collected from stock cultures by means of a sterilized scalpel. A small amount of inoculum was applied to the upper surface of the leaf and spread on it with a sterile brush.

After incubation the cultures were kept free from contamination by air-borne spores. Lamp glasses could only be used in dry weather; during wet weather the rust pustules became mouldy.[1] The cultures kept longer and better if the spore-proof cylinders were made with a special watering place in order to avoid the necessity of lifting the cylinder to water the pot. The cylinder had to be forced deeply into the soil and, during dry weather, it was necessary to see that the cellophane did not become torn. In order to prevent contamination of the cylinders by spores of *Erysiphe* and saprophytes the covers were autoclaved every time new inoculations were made. Under Portuguese conditions, particularly in Lisbon, care was necessary to keep out ants. Stock cultures were renewed every 3 weeks.

When the English physiologic races and some of the Spanish physiologic races had to be transported from England to Portugal in December 1934 they were taken on Spratt Archer seedlings cultivated in Knop's agar in test-tubes by the technique of Ward (1902) and Mains (1917). Cultures were also stored in a refrigerator in a desiccator in which the relative humidity was 50% as advised by Peltier (1925) and Newton & Johnson (1932).

(4) Differentiation of the physiologic races

(a) *Differential hosts.*

Differentiation of physiologic races of *P. anomala* was made chiefly on the basis of Hey's selection of barleys (1932).

Aegyptische 4-zeilige Sommergerste, selected by Gassner & Straib, and used by Ronsdorf (1934), was also tested.

Seven of Mains's differential barleys have also been used.

The following is the list of all barley varieties used as differential hosts:

Hey:
      Hordeum hexastichum eurylepis
      Hordeum hexastichum recens
      Hordeum vulgare speciale
      Breustedts Schladener
      Friedrichswerther Berg Wintergerste
      Australische Recka
      Samaria 4-zeilige
      Hordeum vulgare pallidum (Sudan)
      Lichtis Lechtaler
      Ackermanns Bavaria

Gassner & Straib: Aegyptische 4-zeilige Sommergerste

Mains:
      Featherston C.I. no. 1120
      Oderbrucker C.I. no. 940
      Malting C.I. no. 1129
      Hanna C.I. no. 906
      Quinn C.I. no. 1024
      Bolivia C.I. no. 1257
      Juliaca C.I. no. 1114

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[1] *Hyalopus* sp. (*H. parasitans* B. & C. (?)) was the most common mould on the cultures. *Cephalothecium roseum* Corda and *Fusarium* sp. were also recorded.

Seeds of these barleys were kindly supplied to Prof. F. T. Brooks by Dr Hey and Dr A. B. Mains.

The first experiments were carried out with the original seed, but later seeds from crops grown at Cambridge and Lisbon were used.

With one exception all the barleys reacted more or less as pure lines to our physiologic races. Later investigations showed that Friedrichswerther Berg Wintergerste had a small amount of foreign seed mixed with it which proved to be *Hordeum distichum.*

(b) Infection types and reaction classes.

The classification of the reaction classes adopted in the course of this work is the one described by Mains & Jackson (1926) and subsequently used by Waterhouse (1927), Mains (1930), Mains & Martini (1932), Hey (1932) and Ronsdorf (1934, 1935):

Type 0 = *Extremely resistant.* No uredo pustules. Infection only visible as small points or flecks, sometimes in rings, but chlorotic areas do not become darkened.

Type 1 = *Very resistant.* Uredo pustules minute, few. Infection chiefly visible as necrotic flecks without pustules.

Type 2 = *Moderately resistant.* Uredo pustules rather small. Chlorotic and necrotic flecks predominating, usually without pustules.

Type 3 = *Moderately susceptible.* Uredo pustules of moderate size or large, more or less numerous. Generally some chlorosis.

Type 4 = *Extremely susceptible.* Uredo pustules large, numerous and generally confluent. True hypersensitiveness absent, but a little chlorosis when unfavourable conditions exist.

Type X, as described by Stakman & Levine (1922) for *P. graminis tritici*, was added to Mains's symbols because it seemed to be the only means of representing the rather heterogeneous reaction often shown on Bolivia and Juliaca barleys:

Type X = *Heterogeneous reaction.* Uredo pustules very variable, apparently including all types and degrees of infection often on the same blade; no mechanical separation possible; on reinoculation small uredinia may produce large ones and vice versa.

"Variable" or "intermediate" types of reaction such as $0+1-$, $1+2-$, $2+3-$, $3+4-$, $1\cdot2$, $2\cdot3$, etc., and $1+$ or $1-$, $2+$ or $2-$, etc., were also used when the type of reaction was not sharply defined. However, on final readings a fixed type was allotted whenever possible.

(c) Inoculation and incubation.

Testing of pure stock cultures was carried out on seedlings growing in good loam soil in small pots.

The whole range of Hey's and Mains's barleys was usually tested at the same time with any one culture. When possible more than one culture (often as many as five) were tested on the same day in order to make the comparison of the reactions of different cultures as easy as possible. The method of inoculation was the same as for the maintenance of the stock cultures. Care was taken to avoid heavy spore inoculation, which in some susceptible varieties, as pointed out by Hey (1932), causes abnormal necrotic zones. The incubation of the whole set of differential hosts, inoculated with the same

culture, took place under a small glass frame for 48 hr. After incubation the pots were transferred to the benches in the greenhouse and were adequately watered.

In winter, under Portuguese conditions of light and at a mean temperature of 10-14° C. (Lisbon), flecks were visible in 4 days in susceptible varieties and pustules were open on the eighth or ninth day after inoculation. Under English winter conditions at Cambridge with very low light intensity and in the cold compartment of the greenhouse (mean temp. 5·3° C.), the flecks were sometimes visible only on the sixth or seventh day and pustules were open about a week later. Under the same conditions of light but in the warmer house (mean temp. 14·8° C.) pustules were out on the ninth or tenth day. With high light intensity and mean temperature of 15-19° C. flecks were visible about 3-4 days after inoculation and pustules were coming out on the eighth or ninth day. During summer, when the temperature did not exceed 22-26° C., pustules were often open on susceptible varieties within 6 days. When the weather was very hot—27° C. or over—the pustules sometimes began to appear on the sixth day, but the reaction was not fixed until some time later. Generally speaking these results are very similar to those obtained by Hey.

Determinations of the reaction type were made three times: the first observation was made when pustules appeared, the second 3 days later, and the third within 2 or 3 days to confirm the previous observations. As a rule the first two readings are adequate.

(d) *Method of determining new physiologic races and frequency of tests.*

Every single-spore culture was tested on the differential barleys as soon as possible after isolation. When the reactions of the first set of inoculations coincided exactly with those of any of the physiologic races already studied the culture was discarded.

On the other hand, if the reactions of a particular culture did not agree closely with those of known physiologic races a new test was carried out on the whole range of hosts side by side with tests of those cultures closely related to it. Care was then taken to keep the sets under exactly the same conditions, and the readings of the reactions were taken at the same time. If the new culture could not be identified with any of the others it was kept in order to determine the reaction variations. The cultures could be tested practically throughout the year. In Cambridge, however, during dull weather from November to February the reactions sometimes became confused; in Portugal, during very hot summer months, if the barleys were not kept under the cooled frames for at least 48 hr. after inoculation, infection was very irregular and often only a few pustules could be observed. Such results were ignored.

The number of times the individual tests were made varied somewhat. The early cultures have been tested since the end of 1933, while the more recent ones, some of which were isolated during the spring and summer of 1936, have been tested only twice. No account is given of any physiologic race which was not tested at least twice on all Hey's and Mains's barleys.

EXPERIMENTAL RESULTS

(1) *Reactions of the physiologic races isolated*

From the eighty-two cultures of *P. anomala* established from uredosporic material, eleven new physiologic races were isolated. The extreme reactions of each of these new races are summarized in Table I, and the

provisional numbers of 12 to 22 are ascribed to them. None of the races previously described by Hey (1932), Ronsdorf (1934, 1935) and Mains (1930) was found.

One of the races, 12, was found to be widely distributed in England, Portugal and Spain. The remaining ten were localized. Races 13, 14, 15, and probably 18,[1] were found on English material, races 18, 19 and 20 on Portuguese material, and races 16, 17, 21 and 22 on Spanish material.

(2) *Differences between physiologic races*

Two of the new races, 16 and 17, resemble race 1 closely as regards their reactions on Hey's differential hosts. As the reactions of this last race on Mains's and Aegyptische 4-zeilige Sommergerste barleys are not known, distinction between race 1 and races 16 and 17 was only possible on Samaria 4-zeilige. The differences on this barley are slight, the reactions being $3-2$ for race 1 and $3+4-$, 4 for races 16 and 17. However, differences as small as these have already been adopted by Hey to differentiate races 4 and 5. Further evidence to maintain them as separate races is based on the reactions on Quinn, which is immune to race 17 ($-$) and only resistant or moderately resistant ($1, 3-$) to race 16.

Race 11, isolated by Ronsdorf from North American material, Michigan, and tested only on Hey's barleys, is somewhat similar to race 12, but is easily differentiated on *Hordeum vulgare speciale* and on Breustedts Schladener, both with a reaction type $3-2$ for race 11 and $4-$, 4 for race 12.

Race 13 can be differentiated from race 12 and from race 11 by the reactions on Australische Recka ($3+4-$, 4 for race 12, 1, 2 for race 13 and 3 for race 11). Race 8, tested only on Hey's barleys, showed reactions very similar to those of race 20. Differentiation was established chiefly by the reactions on Samaria 4-zeilige (2–3 for race 8, 4 for race 20). Lichtis Lechtaler and Ackermanns Bavaria are also somewhat more susceptible to race 20 (reaction 2) than to race 8 (reaction 1).

Races 18, 19, 20, 21 and 22 are more sharply distinct from the races already described by Hey and Ronsdorf. They form a group of which the main feature is high resistance shown by Hey's barleys, *H. vulgare speciale* and Breustedts Schladener (reaction 0 to $1+$), and by Mains's barleys, Featherston, Oderbrucker and Malting (reaction 0 to 2).

Race 19 is clearly differentiated from the other four on Friedrichs-werther Berg Wintergerste, which is highly susceptible to this race

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[1] A culture established from material sent from Reading and only tested once reacted apparently as race 18. The culture was lost soon after the first test.

Table I. *Extreme reactions of eleven new physiologic races established from uredosporic material of P. anomala*

Year → Physiologic races	1934-6 12	1934-6 13	1934-6 14	1934-6 15	1934-6 16	1934-6 17	1935-6 18	1936 19	1936 20	1936 21	1936 22
Country	England Portugal and Spain	England	England	England	Spain	Spain	Portugal	Portugal	Portugal and Spain	Portugal and Spain	Spain
Barleys:											
Aegyptische 4-zeilige Sommergerste	3+4-, 4	3+4-, 4	2, 3+	3, 4	0, 2-	0, 1+2-	4	4	4-	2-, 3	4-, 4
Hordeum hexastichum eurylepis	4	4	4	3, 4	4	4	4	3+4-	4-	4-	4
Hordeum hexastichum recens	4	4	3+4-, 4	3+4-, 4	4	4	4	4	4-	3, 3+	4
Hordeum vulgare speciale	4	4	1+, 4X	0, 1	0, 2	0, 2	0, 1	0, 1	0	0	0, 1
Breustedta Schladener	4-, 4	4	1, 4-	0, 1	0, 2	0, 2	0, 1	0, 1	1	0	1+
Friedrichswerther Berg Wintergerste	3+4-, 4	3+4-, 4	2, 4-, X	0, 1-2*	0, 2	0, 2	0, 1	4	1	0	1+
Australische Recka	3+, 4	1, 2	2, 4	3, 4	0, 2	0+, 2	4	4	4-, 4	3-4*	4
Samaria 4-zeilige	3+4-, 4	4	4-, 4	3+4-, 4	4	4	4	4	4	4	2-, 2
Hordeum vulgare pallidum (Sudan)	4	4	3-, 3	4	4	4	4	4	4	4-	4
Lichtia Lechtaler	3-, 4-	3, 4-	3+4-, 4	4	3+4-, 4	3+4-, 4	4	2+3-	2	4-	4
Ackermanns Bavaria	3-, 4	3, 4-	3, 3+4-	3+4-, 4	3+4-, 4	3+4-, 4	4	2	2	4-	4
Featherston C.I. no. 1120	4	4	X, 2,3+4-	1, 2+3-	1+2-	0, 2	0, 1+	1+, 2	2	0	1+2-
Oderbrucker C.I. no. 940	4-, 4	4	1, 3+	0, 1-2*	0, 2	0, 2	0, 1+	1	2-	0	1, 2
Malting C.I. no. 1129	4-, 4	4	1+2-, 3+	0, 1	0, 2	0, 2	0, 1+	1	1+	0	1, 2
Hanna C.I. no. 966	4	4	4-, 4	3+4-, 4	4	4	4	4	4	4	4
Quinn C.I. no. 1024	3, 4	3, 4	2, 3+4-	1, 2+3-	2, 3	0	4	4	4	3+4-	1-, 2-
Bolivia C.I. no. 1257	4-, 4X, 4	4X, 4	3+4-, 4X	3, 4, X	3X, X	3, X	3+4-	4	3	2, 3	4
Juliaca C.I. no. 1114	4-, 4X, 4	4X, 4	3+4-, 4X	X, 4-, 4	X	X, 4	4	4	4-	3	X, 4

* = reaction range of 1 to 2 or 3 to 4.

(reaction 4), while it proves to be highly resistant to races 18, 20, 21 and 22 (reaction 0 to 1 +). Race 22 is also differentiable from the other three on Quinn, which is resistant to this race (reactions 1 − and 2 −) and is susceptible to the other races, with reaction types 3 + 4 − and 4.

The remaining three races (18, 19 and 21) are still more closely related; their distinction, however, is possible on Aegyptische 4-zeilige which is moderately resistant (2 − to 3 −) to race 21 and susceptible to races 18 and 20. Finally, these last two can be differentiated on Lichtis Lechtaler, susceptible to race 18 (reaction 4) and moderately resistant (reaction 2) to race 20.

Race 14 was rather inconstant as regards its reactions. There was always a great deal of variation on several of the most important differential barleys. Reaction types with this race varied as much as from 1 + to 4 and X on *H. vulgare speciale*, 1 to 4 − on Breustedts Schladener, and 2 to 4 − and X on Friedrichswerther Berg.

(3) *Mutation in physiologic race* 14

Colour mutation in *P. anomala* was noticed in June 1934. During a test of race 14 on the standard barleys, several orange pustules were observed amongst brown ones on *Hordeum vulgare pallidum* (Sudan). The orange pustules were not attributed to mutation at the time. In July, when new tests were being carried out with the same race, more orange pustules were observed on the same barley. Single-spore isolations from these orange pustules on *H. vulgare pallidum* were then made on Spratt Archer, and from these stock cultures were obtained. These cultures maintained the orange colour although it was not quite as bright as on the original barley. In August a comparative inoculation test was made on the differential hosts. It was then seen that the colour mutation was accompanied by mutation in pathogenicity, for on some of the barleys the reactions were different from those of race 14 (see Table II). This mutant has been numbered physiologic race 23.

During these preliminary experiments in July and August, light orange pustules appeared several times in other tests with physiologic race 14 on *H. vulgare pallidum* amongst the normal brown ones. From one of these brown pustules on *H. vulgare pallidum* a new single-spore culture was established and maintained on this barley in order to find out whether mutation would occur in a culture originating from a spore which had kept normal under conditions that induced mutation in others. In a later transfer several orange pustules again appeared. From September 1934 until May 1935 no more light-coloured pustules appeared in any of

the cultures of race 14. Then in May and June 1935, when new tests were being made with this race on *H. vulgare pallidum* in Portugal, under spore-proof conditions, further orange and a few light yellow pustules appeared. New single-spore cultures were established from the yellow ones.

Table II. *Reactions of parent race (no. 14) and its mutant derivative (no. 23)*

| Barleys | Extreme readings | | Comparative test | | | |
| | Parent | Mutant | Parent race 14 | | Mutant race 23 | |
			25. ix	20. xi	25. ix	20. xi
Aegyptische 4-zeilige Sommergerste	2, 3+	2-, 3, 3X	3	2	3	2-
Hordeum hexastichum eurylepis	4	3+, 4-, 4	4	4	4	3+
Hordeum hexastichum recens	3+4-, 4	4	4	4-	4	4
Hordeum vulgare speciale	1+, 4X	0, 1-	3	4X	0·1-	0
Breustedts Schladener	1, 4-	0, 1	4-	X	0	0
Friedrichswerther Berg Wintergerste	2, 4-, X	0, 1	4	X	0	0
Australische Recka	2, 4	4-, 4	2	4	4	4
Samaria 4-zeilige	4-, 4	4	4	4	4	4
Hordeum vulgare pallidum (Sudan)	3-, 3	4	3	2	4	4
Lichtis Lechtaler	3+4-, 4	3+4-, 4	3+4-	3+4-	4	3+4-
Ackermanns Bavaria	3, 3+4-	4	3+4-	3	4	4
Featherston C.I. no. 1120	2, 3+4-X	0	3+4-	X	0	0
Oderbrucker C.I. no. 940	1, 3+	0, 0·1	1	3+	0	0
Malting C.I. no. 1129	1+2-, 3+	0	1+2-	3+	0	0
Hanna C.I. no. 906	4-, 4	4	4	4	4	4
Quinn C.I. no. 1024	2, 3+4-	2+, 4	2+	3-	3-	3+4-
Bolivia C.I. no. 1257	3+4-, 4, X	0, 2	3+4-	3+4-	1+	0
Juliaca C.I. no. 1114	3+4-, 4, 4X	4, 4X	3+4-	3+4-	4	4

A new comparative test between race 14, the first orange mutant (race 23), and the light yellow culture was then carried out. The light yellow mutant proved to be very similar to, if not identical with, the orange mutant as regards pathogenicity and was therefore classed as physiologic race 23.

The orange and yellow mutants appeared several times until about the middle of October 1935. After that, although some light-coloured pustules were observed, they were not stable, for when subcultures were made brown pustules always appeared.

It seemed that temperature probably played an important part in the occurrence of these changes. In order to test this an experiment was set up early in 1936 in which seedlings of *H. vulgare pallidum*, growing in pots, were inoculated on the same day with uredospores from culture 14. After an incubation period of 24 hr. the pots were divided into two sets. One set was left on the bench of the greenhouse where the temperature

range was 15–25° C. maximum to 8·5–14·5° C. minimum. The other set was transferred to an incubator with a glass door at the top, the temperature being maintained at 25–27° C. The seedlings in the greenhouse never showed any light-coloured pustules, while on those kept in the incubator several orange pustules appeared among the brown ones. Isolations were made from these orange pustules, but reversion to the brown colour always occurred.

Occasionally some of the other physiologic races, namely, 15, 16, 17 and 18, produced a very few orange pustules on *H. vulgare pallidum* as well as on Bolivia and Juliaca. However, all attempts to obtain new colour mutants failed.

The above results seem to confirm Stakman's (1930) assertion that the "frequency of mutation can be influenced by environmental conditions, such as amount and kind of nutrients and temperature".

In the mutation of race 14 the parts played by the nature of the host and the temperature seem quite definite. As yet, it is not possible to determine whether temperature has a direct effect or whether it acts indirectly by producing changes in the host.

Another important factor to be considered is the degree of instability of the physiologic race itself.[1] In the mutations that have been observed under controlled conditions, it appears that this phenomenon of reaction instability plays an important part. All four mutations found by Stakman *et al.* (1930) resulted at different times from the same culture of race 1 of *P. graminis tritici*. The mutant of *P. glumarum tritici*, reported by Gassner & Straib (1932), occurred on thirty-four cultures of one strain of the rust. Cotter & Levine's (1932) colour mutant of *P. graminis secalis* was observed simultaneously in two cultures of the same origin. Roberts (1936) described a mutant of race 66 of *P. triticina* which arose from a culture apparently unstable in reaction. The culture of physiologic race 14 of *P. anomala*, from which the present mutant was produced, was very inconstant in reaction. Another interesting point is the constancy with which the mutation took place on one particular barley, *Hordeum vulgare pallidum* (Sudan). The influence of the host as a determinant in the occurrence of mutation in this culture seems so well marked that it may support another interpretation of Ward's (1903) "bridging host" theory. The "bridging host" might in fact induce persistent changes in pathogenicity.

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[1] This instability may concern only some strains of one race, as Roberts (1936) found in the case of race 66 of *P. triticina*. It is necessary to keep in mind, as Johnson *et al.* (1934) emphasize, that different cultures of the same physiologic race may be genetically different.

(4) *New physiologic races obtained from aecidia*

Craigie (1927–31) carried out the pioneer work on the relation of the pycnidia to the aecidia. For several rusts new physiologic races have been produced by hybridization or self-fertilization, notably by Waterhouse (1929), Newton *et al.* (1930*a*, *b*, 1932) and Murphy (1935).

No records of similar investigations on *P. anomala* have hitherto been published. Early in 1936 I succeeded in producing aecidia on species of *Ornithogalum* with sporidia of *P. anomala,* as will be described in detail in another paper. From the aecidia new physiologic races were obtained by segregation and hybridization which will now be described.

(a) *Material and methods.*

The *Ornithogalum* plants, grown in pots, were inoculated with sporidia and were then kept under cheese cloth frames or cellophane cylinders. The technique of Craigie (1931) or of Newton & Johnson (1932) was used.

For purposes of hybridization, leaves with a single haploid infection were chosen. Usually not more than one infection per plant or per group of plants was selected. These infections were allowed to develop for not less than 3 weeks to make sure that no self-fertilization had taken place. Only then were these pustules set apart to be hybridized.

Crossing of two physiologic races was done by transferring spermatial nectar from one infection to another and vice versa with a sterile needle or brush. Unfortunately, material for hybridization, under the conditions described above, was not obtained at the same time for all the physiologic races with which infections on *Ornithogalum* were obtained.

On the other hand, in some of the physiologic races self-fertilization could easily be brought about by mixing spermatia from different pustules. All but one of the new physiologic races obtained through the aecidial stage were produced in this way.

(b) *Pure cultures from aecidia.*

Previous workers have shown that in the heterozygous races, distinct physiologic races can be obtained from different aecidia of a single infection; care was therefore taken to avoid mixing the spores of separate aecidia.

The first method attempted to establish pure cultures from aecidia was that described by Newton & Johnson (1932) for the aecidia of *P. graminis*. But, as *Aecidium Ornithogalum* does not form a cupulate body outside the leaf, it was almost impossible to detach the aecidia from the leaves by means of forceps. Even when that was successfully achieved and chains of aecidiospores obtained, these showed very poor germination if compared with the freshly ripened spores.

Mass inoculations from a single aecidium are not advisable with this rust because later experiments showed that more than one physiologic race could be isolated from the spores of a single aecidium. Accordingly, a new technique was devised and tried with encouraging results.

One aecidial pustule was chosen and all the aecidia which were already open were covered with a layer of vaseline and only a single unopened aecidium was left uncovered.

This was done to prevent any possibility of the spores becoming mixed. As soon as the peridium of the uncovered aecidium split the spores were picked up with a sterile needle and scattered on a sterile slide. The aecidium was then covered with vaseline to prevent its spores from becoming mixed with those of aecidia which developed subsequently. By this method it was possible to isolate spores from more than one aecidium in a single infection without danger of mixing them.

From the aecidiospores on the slides, single-spore inoculations were made on Spratt Archer seedlings by the usual technique. When pustules appeared on the barley pure cultures were established.

The physiologic races from which aecidia were obtained are nos. 12, 13 and 23. Abundant material was obtained from nos. 12 and 13, but with no. 23 only a few infections resulted.

Physiologic races obtained from each source are described separately.

(aa) Aecidia from infections obtained with physiologic race 12

Infection I. Single-spore cultures were attempted from twenty-one aecidia but from only fifteen of them could cultures be established. From the progeny of these fifteen aecidia six physiologic races were identified. Two proved to be physiologic races 12 and 13 respectively. Another was race 16, or a very similar one. The other three were new races, and these are described in Table III under the numbers 24, 25 and 26.

In thirteen cases out of the fifteen the cultures resulting from spores of the same aecidium behaved as the same race, but in the two other aecidia (nos. 4 and 21) more than one physiologic race was isolated. From aecidium no. 4 one culture could be identified with the mother culture (race 12) and two others with race 24. From aecidium no. 21 races 12 and 26 were differentiated. The frequency with which the races appeared in the progeny is as follows:

Physiologic races segregated by race 12:	12	24	13	25	16	26
No. of aecidia in which the race was found:	6	4	3	2	1	1

Infection II. From this infection eleven aecidia were selected and it was possible to establish cultures from nine of them. The cultures so obtained were found to belong to physiologic races 12, 19 and 24 in the proportion of 7 : 1 : 1.

Infection III. In this infection isolations from twenty-eight aecidia were attempted, but cultures were obtained from seven only. Four of the aecidia developed as race 12, one as race 24 and the other two as a new race which was numbered 27.

The heterozygous condition of physiologic race 12 is clearly revealed by the progeny of the thirty-one aecidia produced in three infections. In

this progeny eight different physiologic races were identified. Four of them proved to be already known, or at least they gave the same reactions on our range of hosts as four of those found in nature. These four were 12, 13, 16 (or a variation of 16) and 19. The other four races were new and they were numbered 24, 25, 26 and 27.

(bb) Aecidia from infections obtained with physiologic race 13

Infection I. Isolations were attempted from twenty-three aecidia and single-spore cultures were obtained from nineteen. These proved to belong to five physiologic races. Three reacted as races 12, 13 and 22; the other two proved to be new and were numbered 28 and 29. Their reactions are given in Table III.

Table III. *Reactions of physiologic races 24–29*

Physiologic races ...	24	25	26	27	28	29
Barleys:						
Aegyptische 4-zeilige Sommergerste	0	4	4, 4X	4 –	4	4, X
Hordeum hexastichum eurylepis	4	4	4	4	4	4
Hordeum hexastichum recens	4	4	4	4	4	4
Hordeum vulgare speciale	4	4 –, 4	0	4	4	4
Breustedts Schladener	4	0, 1	0	4 –, 4	0, 1 +	4
Friedrichswerther Berg Wintergerste	4	0, 1	0, 1 –	4	0, 1	4
Australische Recka	4	4	4	0, 0·1	4	2 +
Samaria 4-zeilige	4	4 –, 4	4	4	4	4
Hordeum vulgare pallidum (Sudan)	4	4	4	4	4	2
Lichtis Lechtaler	3, 3 +	3 + 4 –	2 –, 2 + 3 –	2	3 + 4 –	3 + 4 –
Ackermanns Bavaria	3, 3 +	3 + 4 –	2 –, 2 + 3 –	2, 2 +	3 + 4 –	4 –
Featherston C.I. no. 1120	4	4	1, 1 +	4	4	4
Oderbrucker C.I. no. 940	4	4	1	4	4	4
Malting C.I. no. 1129	4	4	0, 1	4	4	4
Hanna C.I. no. 906	4	4	4	4	4	4
Quinn C.I. no. 1024	2 + 3 –, 3	1·2, 2	2	2, 2 +	4	1 + 2 –
Bolivia C.I. no. 1257	4, X	4 –, 4X	4	4	4	4
Juliaca C.I. no. 1114	4 –, X	4, X	4	4	4	4
No. of aecidia in which the race was found:	6	2	1	2	1	3

Only one physiologic race was found in each aecidium. The proportion in which the races appeared is as follows:

Physiologic races segregated by race 13:   13   22   12   29   28
No. of aecidia in which the race was found: 9   4   3   2   1

Infection II. Cultures were obtained from ten aecidia, and physiologic races 13, 22 and 24 were identified in the proportion of 4 : 4 : 2.

(*cc*) *Aecidia from infections obtained with physiologic race* 23 (*yellow*)

Only one infection, fertilized with spermatial nectar of the same origin, was available for aecidiospore isolation. Eleven aecidia from this infection were selected and in their progeny four physiologic races were segregated, viz. nos. 12, 19, 20 and 22.

All the cultures from aecidiospores showed only brown uredospore pustules. Colour segregation, as described by Newton & Johnson (1932) in the progeny of a colour mutant of *P. graminis tritici*, was not detected in the limited number of isolations attempted.

(*dd*) *Results of a reciprocal cross between physiologic races* 13 *and* 23

Three crosses between haploid infections of race 13 and haploid infections of race 23 were attempted. From the two hybridizations so obtained cultures were made and tested in the usual way. When race 23 was fertilized with spermatia of 13 the aecidiospores which were isolated gave rise to a new physiologic race described in Table IV as no. 30. From the reciprocal cross physiologic race 30 was found in five aecidia and race 18 in two others.

Table IV. *Reactions of races* 13 *and* 23 *and of their reciprocal crosses*

| | 13→23 | 13←23 | | Crossed races | |
Physiologic races ...	30	18	30	13	23
Barleys:					
Aegyptische 4-zeilige Sommergerste	0, 1 –	4, 4X	0, 0·1	3 + 4 – , 4	2 – , 3, 3X
Hordeum hexastichum eurylepis	4	4	4	4	3 + 4 –
Hordeum hexastichum recens	4 – , 4	4	4	4	4
Hordeum vulgare speciale	0	0, 1	0·1 – , 1	4	0, 1 –
Breustedts Schladener	0	0, 0·1	0, 1 –	3 + 4 – , 4	0, 1
Friedrichswerther Berg Wintergerste	0, 1	0, 1	0, 1 +	1, 2	0, 1
Australische Recka	3 + 4 – , 4	4 – , 4	4	4	4 – , 4
Samaria 4-zeilige	4	4	4	4	4
Hordeum vulgare pallidum (Sudan)	4	4	4	4	4
Lichtis Lechtaler	4	4	3 + 4 – , 4	3, 4 –	3 + 4 – , 4
Ackermanns Bavaria	4	4	3 + 4 – , 4	3, 4 –	4
Featherston C.I. no. 1120	1	1	1	4	0
Oderbrucker C.I. no. 940	0, 1	1	1	4	0, 0·1
Malting C.I. no. 1129	0, 1 +	1	0, 2 –	4	0
Hanna C.I. no. 906	4	4	4	4	4
Quinn C.I. no. 1024	1	4	0, 1 –	3, 4	2 + , 4
Bolivia C.I. no. 1257	4, 4X	4, 4X	4, 4X	4, 4X	0, 2 –
Juliaca C.I. no. 1114 ˙	4, 4X	4 –	4, 4X	4, 4X	4, 4X
No. of aecidia in which the race was found:	6	2	5	—	—

Behaviour in this hybridization, in which one of the reciprocal crosses gave rise to two physiologic races, is somewhat different from the results

of Newton & Johnson (1932) with *P. graminis tritici*. These authors stated that in some reciprocal crosses cytoplasmic inheritance could be demonstrated by the origin of different physiologic races, but in no case was more than one race isolated from each infection. Goldschmidt (1928) postulated that for *Ustilago violacea* Pers. such genetic characters are cytoplasmic. On this basis the above results with *P. anomala* could only be explained by supposing that more than one hypha had been fertilized by different spermatia. If so, the cytoplasms might carry pathogenic factors capable of modifying the general type of pathogenicity dependent on the hybrid nucleus.

(5) *Biotypes of physiologic races* 12 *and* 13

During our experiments a large number of cultures, identified with races 12 and 13, showed slight but constant differences in reaction. These differences were observed chiefly on Australische Recka, Lichtis Lechtaler and Ackermanns Bavaria. The reactions of some of these cultures were compared under the same conditions.

Thus, from race 12 cultures 35, 54 and 71 were inoculated at the same time. They were incubated together and maintained on the same bench of the greenhouse. The following results were obtained:

	Cultures		
	54	35	71
Australische Recka	3, 3 +	4	3 + 4 −
Lichtis Lechtaler	4	3 + 4 −	2 + 3 −
Ackermanns Bavaria	4	3 + 4 −	2 + 3 −

Also ten single-spore cultures from an aecidium, which apparently reacted as race 13, were compared in the same way. These cultures were numbered "13, aec. 4, 1 to 10", but will be referred to as 1, ..., 10. It was not possible to inoculate all of them at the same time, but two sets of inoculations were made, using five cultures each time, and an interval of 2 days was allowed between the two series. During the time of the experiment there was no significant change of temperature to influence the reactions. The results confirm the preliminary observations, for in both sets of inoculated barleys sharp differences were observed. The reactions on the three barleys were as follows:

	Cultures									
	3	7	2	4	6	8	9	1	5	10
Australische Recka	0	0, 1	1	1	1 +	2	2	2 +	3 −	3
Lichtis Lechtaler	3	4	2 +	3	3	3 −	2	3 −	3 + 4 −	3 +
Ackermanns Bavaria	3	4	2 +	3	3	3	2 +	3 −	3 + 4 −	3 +

It is difficult to decide whether some of these biotypes ought to be considered as belonging to races 12 or 13. According to the present definition of a physiologic race, culture no. 3 (reaction type 0) on Australische Recka is different from culture no. 1 (reaction type 2 +) and is even more distinct from culture no. 10 (reaction type 3). However, the differences become less evident when the two extremes are compared with the original type culture, which gives a reaction 1, 2. Moreover, considering the reactions of races 12 and 13 on Australische Recka which are respectively 3 +, 4 and 1, 2 and the reactions of the ten biotypes on the same barley, it is possible to construct a continuous series linking together the two races.

This hypothesis, suggested by the experiments, is corroborated by the existence of very closely related races of *P. graminis* and *P. triticina.* Stakman & Levine (1935) call attention to this in the "Analytical Key for the Identification of Physiologic Forms of *P. graminis tritici*": "It is well to keep in mind that in some cases what is designated as a form may in reality comprise several closely related biotypes. Furthermore, some forms (races) are so closely related that it is difficult to distinguish between them. It appears that there is an indefinite number of biotypes of *P. graminis tritici* many of which differ from each other almost imperceptibly."

A NEW ARRANGEMENT OF DIFFERENTIAL HOSTS FOR THE PHYSIOLOGIC RACES OF *P. ANOMALA*

The reaction tests of the nineteen new physiologic races described above were tried on Hey's differential set, on some of Mains's differential barleys and on Aegyptische 4-zeilige Sommergerste. The results showed that a new arrangement of the standard differential hosts selected by Hey was necessary to make it efficient.

In Hey's collection of barleys some hosts are duplicated at least as far as concerns the reactions of the races which are already known. On the other hand, it fails to show differences between those of our races which are differentiable on other hosts. Thus Lichtis Lechtaler and Ackermanns Bavaria both react in the same way to all the physiologic races. *Hordeum hexastichum eurylepis* and *H. hexastichum recens* react in a very similar way to them. These two barleys are susceptible (reaction type 3 + 4 − or 4) to all the races except races 4, 5 and 6, to which they are moderately resistant (reaction type 2–3 or 2). Moreover, they are of no value in differentiating races 4, 5 and 6 from each other.

On the contrary, Gassner & Straib's barley Aegyptische 4-zeilige Sommergerste, added by Ronsdorf to Hey's selection, proved to be the key to differentiation between some physiologic races, and it is quite indispensable in differentiating race 24 from 12; with these two races it gives reaction types 0 and $3+4-$, 4, respectively.

Two of Mains's barleys also proved to be of great value. Quinn C.I. no. 1024 was the only barley on which physiologic races 16 and 17 could be differentiated. It gave a reaction type 0 with the latter and 2–3 with the former. Also, race 18 could be distinguished from race 22 on this barley, the former giving reaction 4 and the latter $1-$, $2-$. Lastly, Quinn C.I. no. 1024 was useful in distinguishing races 20 and 22. Bolivia C.I. no. 1257 was the only barley on which races 21 and 23 could be differentiated. Race 21 gave a reaction 2, $3-$ and race 23 gave a reaction 0, $2-$. Unfortunately, this barley is of little value for differentiating the other races, since it often shows the heterogeneous reaction X. However, it must be kept in the differential set until a better variety is found.

For the purpose of differentiating the thirty physiologic races of *P. anomala* Rost. now known the following barleys are suggested:

> Breustedts Schladener
> *Hordeum vulgare speciale*
> Friedrichswerther Berg Wintergerste
> Australische Recka
> Lichtis Lechtaler
> Samaria 4-zeilige
> *Hordeum vulgare pallidum* (Sudan)
> Aegyptische 4-zeilige Sommergerste
> Quinn C.I. no. 1024
> Bolivia C.I. no. 1257

Oderbrucker C.I. no. 940 may also be kept as a subsidiary variety for differentiating the two groups of races, as suggested by Ronsdorf, which are differentiated by reactions 0–1 and 3–4.

Although this new arrangement of differential hosts makes possible a much better differentiation of the races much work remains to be done on this subject. First it seems necessary to carry out an investigation on a large collection of barleys, using not only the well-differentiated physiologic races, but also certain strains, now grouped under the same race which give slight, but constant, different reactions. Such slight differences were emphasized for cultures of races 12 and 13. These differences in reaction have been regarded as merely satellite variations, but in future they may prove to be due to different physiologic races not differentiable on the basis of our actual host range.

Bruens Neuzucht 25 was chosen by Stakman *et al.* (1935) as a differential host on the basis of an analysis of Hey's results (1932). It gave reaction type 4 with races 1, 2, 3, 4 and 5 and reaction 1 with race 6. This barley should be tested with all the available races to ascertain its real value as a differential host.

The six barleys, Gold, Flinn, California Feed, Stavropol, Chile and Odessa, used by Brown(1931) for differentiation of four Canadian physiologic races of brown rust of barley, should also be tested in order to determine whether any of the European races may be identified with the Canadian ones, and to establish the merits of these barleys as differential hosts.

The reaction types of the thirty physiologic races on the new set of barleys are given in Table V and an analytical key is provided in Table VI. In Table V only the extreme readings obtained during the experiments with physiologic races 12–30 are given. For the first eight races, the reactions were taken from Hey's (1932) results on Breustedts Schladener, *Hordeum vulgare speciale*, Friedrichswerther Berg Wintergerste, Australische Recka, Lichtis Lechtaler, Samaria 4-zeilige and *H. vulgare pallidum* (Sudan) at mean temperatures of 18 and 25° C. The reactions on Aegyptische 4-zeilige Sommergerste, Quinn C.I. no. 1024, Bolivia C.I. no. 1257 and Oderbrucker C.I. no. 940 of races 2, 3, 4, 5 and 9 were obtained from Ronsdorf's papers (1934, 1935): on Aegyptische 4-zeilige Sommergerste the reactions were taken by Ronsdorf at mean temperatures of 18 and 25° C. (1934), and on the other barleys the reactions were taken at mean temperatures of 12 and 22° C. (1935).

Reactions of races 9, 10 and 11 on Hey's standard barleys were also obtained from Ronsdorf's papers. The readings were taken at 18 and 25° C. for races 9 and at 26° C. for races 10 and 11.

Owing to differences in temperature at which the experiments have been carried out it is difficult to compare the results in some cases. However, this difficulty could not be overcome, as some of the races were described in different countries under diverse environmental conditions.

DISTRIBUTION AND PREVALENCE OF PHYSIOLOGIC RACES OF
P. ANOMALA IN GREAT BRITAIN, SPAIN AND PORTUGAL

The present account of the distribution of physiologic races of *P. anomala* Rost. must be regarded as a preliminary survey. Material was obtained from Great· Britain, Spain and Portugal, but the localities in these countries were not sufficiently scattered for a comprehensive survey. Thus, with the English material thirty-eight collections were tested but thirty-four of these were collected at Cambridge.

Table V. Pathogenicity of physiologic races of P. anomala Rost. on eleven differential barleys

Physiologic races	Breustedts Schladener	Hordeum vulgare speciale	Friedrichswerther Berg Wintergerste	Australische Recka	Lichtis Lechtaler	Samaria 4-zeilige	Hordeum vulgare pallidum	Aegyptische 4-zeilige	Quinn C.I. no.1024	Bolivia C.I. no.1257	Oderbruker C.I. no. 940
1	0, 1	0, 1	0, 1	1·2	4, 3	2·3, 2	3·4	1, 2·3	0·1, 0	2, 3·2	4
2	3·4	3·4	3	1·2	4, 3	2, 3	1·2	3	1, 0	1·2, 2·3	4
3	3·4	3·4	3·4	1·2	4, 3	2·3, 2	3·4	2, 3·4	0	2	4
4	3·4, 3	3·4, 3	3·4, 3	0·1	4, 3	1, 2	3·4,	1, 2·3	0·1, 0	2	4
5	3·4, 3	3·4, 3	3	1·2, 1	4, 3	1, 2	1·2, 1				—
6	3·4, 3	3·4, 3	3·4, 3	1·2, 1	1, 2·3	2·3, 2	3·4				—
7	0, 1	0, 1	0, 1	1·2	1·2, 2	3·2	3, 3·4				—
8	0, 1	0, 1	0, 1	3·4, 4	1·2, 2	3·2, 3·4	3	3, 3·4	2·1, 0	1·2, 2·3	4
9	3·4	4	3·4	3·2, 3·4	3·4	4·3	3·4				—
10	0·1	0	1	3·2	2·1	3·4	4·3				—
11	3·2	3·2	4·3	3	4·3	3+4 –, 4	4	3+4 –, 4	3, 4	4 –, 4, 4X	4 –, 4
12	4 –, 4	4	3+4 –, 4	3 +, 4	3 –, 4 –	4	4	3+4 –, 4	3, 4	4, 4X	1·3 +
13	1, 4 –	1 +, 4X	3+4 –, 4	1, 2	3, 4 –	4 –, 4	3 –, 3	2, 3 +	2, 3+4 –	3+4 –, 4X	0, 1·2
14	0, 1	0, 1	2, 4 –, X	2, 4	3+4 –, 4	3+4 –, 4	4	3, 4	1, 2+3 –	3, 4, X	0, 2
15	0, 2	0, 2	0, 1·2	3·4	4	4	4	0, 2 –	2, 3	3X, X	0, 2
16	0, 2	0, 2	0, 2	0, 2	3+4 –, 4	4	4	0, 1 +2 –		3, X	0·1 +
17	0, 1	0, 1	0, 2	0 +, 4	3+4 –, 4	4	4	4	4	3 +, 4 –	1
18	0, 1	0, 1	0, 1	4	2+3 –	4	4	4	4	4	2 –
19	1	0	4	4 –, 4	2	4	4	4 –	4	3	
20	0	0	1	3·4	4 –	4 –	4 –	2 –, 3	1 –, 2 –	2, 3	0
21	1 +	0, 1 –	0	4	4	2 –, 2	4	4 –, 4	3+4 –	4	1, 2
22	0, 1	4	1 +	4 –, 4	3+4 –, 4	4	4	2 –, 3, 3X	2+3 –, 4	0, 2	0, 0·1
23	4	4	0, 1	4	3, 3 +	4	4	0	2+3 –, 3	4, X	4
24	0, 0·1	4 –, 4	4	4	3+4 –	4 –, 4	4	4	1·2, 2	4 –, 4X	1
25	0	0	0·1, 1	0, 0·1	2 –, 2+3 –	4	4	4, 4X	2	4	
26	4 –, 4	4	0, 1 –	4	2	4	4	4 –	2, 2 +	4	0, 0·1
27	0, 1 +	4	4	4	3+4 –	4	4	4, X	1+2 –	4	4
28	4	4	0, 1	2	3+4 –	4	2	4, X	1+2 –	4, 4X	4
29	0, 1 –	0, 1	4	3+4 –, 4	3+4 –, 4	4	4	0, 1 –	0, 1	4, 4X	1
30			0, 1 +								0, 1
A Mains's physiologic races		..							0, 2	0, 0·2	3·4, 4
B		..							0, 0·1	0, 0·1	

Table VI. *Analytical key for the identification of physiologic races of* P. anomala *Rost. showing their pathogenicity on ten differential barleys*

Differential hosts and their behaviour	Physiologic races
Breustedts Schladener—resistant to moderately resistant (0·2)	
Hordeum vulgare speciale—resistant to moderately resistant (0·2)	
Friedrichswerther Berg—resistant to moderately resistant (0·2)	
Australische Recka—resistant to moderately resistant (0·2)	
Lichtis Lechtaler—resistant to moderately resistant (1·2)	7
Lichtis Lechtaler—susceptible (3 + 4 −, 4)	
Samaria 4-zeilige—moderately resistant to moderately susceptible (2·3)	1
Samaria 4-zeilige—susceptible (4)	
Quinn C.I. no. 1024—immune (0)	17
Quinn C.I. no. 1024—resistant to moderately susceptible (1·3 −)	16
Australische Recka—moderately resistant to moderately susceptible (3·2)	10
Australische Recka—susceptible (4)	
Lichtis Lechtaler—resistant to moderately resistant (1·2)	8
Lichtis Lechtaler—moderately resistant to moderately susceptible (2 −, 2 + 3 −)	
Quinn C.I. no. 1024—moderately resistant (2)	26
Quinn C.I. no. 1024—susceptible (4)	20
Lichtis Lechtaler—susceptible (3 + 4 −, 4)	
Bolivia C.I. no. 1257—resistant to moderately resistant (0·2 +); yellow pustules	23
Bolivia C.I. no. 1257—moderately resistant to moderately susceptible (2·3)	21
Bolivia C.I. no. 1257—susceptible (3 + 4 −, 4)	
Aegyptische 4-zeilige—resistant (0·1)	30 ✓
Aegyptische 4-zeilige—susceptible (3 + 4 −, 4)	
Quinn C.I. no. 1024—resistant to moderately susceptible (1 −, 2·3)	
Samaria 4-zeilige—moderately resistant (2 −, 2)	22
Samaria 4-zeilige—susceptible (4)	15
Quinn C.I. no. 1024—susceptible (4)	18
Friedrichswerther Berg—susceptible (4)	19
Hordeum vulgare speciale—susceptible (4)	
Quinn C.I. no. 1024—resistant to moderately resistant (1·2, 2)	25
Quinn C.I. no. 1024—susceptible (4)	28
Breustedts Schladener—moderately resistant to moderately susceptible (3·2)	11
Breustedts Schladener—susceptible (3·4, 4)	
Australische Recka—resistant to moderately resistant (0·2)	
Lichtis Lechtaler—resistant to moderately resistant (1, 2·3)	
Hordeum vulgare pallidum—resistant (1·2)	6
Hordeum vulgare pallidum—susceptible (4)	27
Lichtis Lechtaler—susceptible (3 + 4 −, 4)	
Quinn C.I. no. 1024—resistant (0·2 −)	
Bolivia C.I. no. 1257—resistant to moderately susceptible (1, 2·3)	
Hordeum vulgare pallidum—resistant to moderately resistant (1·2)	2
Hordeum vulgare pallidum—susceptible (3·4)	
Samaria 4-zeilige—resistant to moderately resistant (1, 2)	
Aegyptische 4-zeilige—moderately resistant to moderately susceptible (1, 2·3)	5
Aegyptische 4-zeilige—moderately resistant to susceptible (2, 3·4)	4
Samaria 4-zeilige—moderately resistant to moderately susceptible (2, 3)	3
Bolivia C.I. no. 1257—susceptible (4)	29
Quinn C.I. no. 1024—moderately susceptible to susceptible (3, 4)	13
Australische Recka—moderately susceptible to susceptible (3·2, 4)	
Aegyptische 4-zeilige—resistant (0)	24
Aegyptische 4-zeilige—moderately resistant to susceptible (3, 4)	
Quinn C.I. no. 1024—resistant (0, 2)	9
Quinn C.I. no. 1024—susceptible (3 + 4 −, 4)	12

The present results were obtained from seventy-seven collections of *P. anomala*: thirty-eight from five localities in Great Britain: Cambridge, Cardiff, Reading, Truro and Hartford (Northumberland); six from three regions in Spain: Madrid, Jerez de la Frontera and Zaragoza; and the remaining thirty-three from scattered places in Portugal (at or near: Lagos, Alvito, Barreiro, Lisbon, Cintra, Cascais, Leiria, Tomar, Coimbra, Porto, Santo Thirso, Pedras Salgadas, Chaves and Bragança). Eleven new physiologic races were identified and numbered 12–22. The distribution of these physiologic races in the three countries was as follows.

Great Britain:

Race 12 was found at Cambridge. Culture 1 was obtained from the Botany School, and cultures 35, 36, 39, 41, 42 and 44 were obtained from the University Farm.

Race 13 was found near Cardiff and at Cambridge. Culture 32 was obtained from Cardiff, cultures 10–31 were obtained from the Cambridge Botanic Gardens, and cultures 37, 38, 40 and 43 from the University Farm.

Race 14 (culture 34) was obtained from Hartford, Northumberland.

Race 15 was obtained from the Cambridge University Farm (culture 2) and from Truro, Cornwall (culture 33).

Race 18(?), culture 9, was obtained from Reading.

Spain:

Race 12 was found in material sent in 1936 from Jerez de la Frontera (cultures 69 and 70) and from Zaragoza (culture 71).

Races 16 and 17 were found mixed in a sample of material sent in 1933 from Madrid (Campo de Moratalá). Race 15 was identified in cultures 3 and 7 and race 17 in cultures 4, 5, 6 and 8.

Race 21 (culture 68) was obtained in 1936 from material sent from Madrid.

Race 22 (culture 82) was found in another sample sent from Madrid.

Portugal:

A wider survey has been possible in this country during the years 1935 and 1936.

Race 12 was found to be widely distributed over the country from north to south. It was isolated from Lagos (culture 48), Alvito (culture 57), Barreiro (culture 59), Lisbon (cultures 45, 46, 47, 50, 61, 62, 63, 64 and 65), Cascais (cultures 49 and 66), Cintra (culture 60), Leiria (culture

56), Tomar (cultures 51, 52, 53, 54 and 55) and Porto (culture 72).
However, this race was not isolated from any sample sent from the north-eastern part of the country.

Race 18 was isolated from two samples, one from Santo Thirso (culture 58), the other from Pedras Salgadas (culture 67).

Races 19 and 20 were both found in a single collection from Coimbra (cultures 73 and 74).

Race 21 was confined to the north-eastern country of Traz-os-Montes. It was found in samples sent from Chaves (cultures 75–80) and from Bragança (culture 81).

FIELD OBSERVATIONS ON THE OCCURRENCE OF *P. ANOMALA*

There is evidence that *P. anomala* may overwinter in East Anglia either in its uredospore stage or as dormant mycelium. This opinion is founded on field observations made in Cambridge during the years 1933 and 1934. During these winters scattered tiller shoots and seedlings bearing uredo pustules of *P. anomala* were continually found. The rust was abundant on these scattered plants until the end of November. During December it seemed that new infections by means of air-borne spores were more or less checked. In January, February and March only rare, small, single pustules could be detected, chiefly on the leaf sheaths. It seems probable that these scattered infections are sufficient to maintain the uredosporic cycle of this rust during the winter. It is the simplest explanation of the continuation of the rust, since I have never been able to find the aecidial stage of *P. anomala* in England although continuous search has been made for it. April seems to be the most favourable period for the dissemination of the rust spores and in May it is already common.

Under Portuguese conditions, in Lisbon and suburbs, there is no doubt that overwintering of the rust in the uredospore stage commonly occurs. But there the problem is, how does the rust survive the summer? In the hottest months no barleys or tiller shoots can withstand the long drought, and the question remained unanswered until the late summer of 1935. While at Cintra, numerous barley plants were found bearing uredo pustules of this and other rusts. Cintra is high above sea level and the plants were growing freely on the edges of the woodlands on the margins of allotment gardens. The rust was also found at sea level in Colares near brooks and creeks in shady places.

Had a detailed survey been made it is possible that a similar summer distribution of uredospores would have been observed in other places.

The observations at Cintra are significant because they show that the rust may pass the summer successfully in localized cool, sheltered places. As soon as the first rains begin in early autumn new barley plants grow from the fallen grains, providing for the propagation of the rust. From such altitudes the spores are carried by wind to the plains in central Portugal where most of the winter cereals are grown.

This explanation agrees with Mehta's (1933) view on the maintenance of cereal rusts in India. It also seems a more probable explanation than the infection of barley by aecidiospores. Although *Ornithogalum umbellatum* is fairly common in Portugal, no single natural infection of *Aecidium ornithogalum* has been found. In support of this view there is also the fact that near Lisbon the only prevalent physiologic race is no. 12, which from English material proved to be heterozygous. If the aecidial stage developed regularly every year in this region, to maintain the rust through the summer, it would most probably give rise to other physiologic races. However, no other physiologic race could be found either at Cintra, Cascais or Lisbon.

SUMMARY

1. Methods of establishing pure cultures of rusts are discussed and a new spore-proof and insect-proof cellophane cylinder is described.

2. Eighty-two cultures, isolated from collections of *P. anomala* in Great Britain, Portugal and Spain, have been tested for the determination of physiologic races. The barleys used as differential hosts were those used by Hey and Mains and also Aegyptische 4-zeilige Sommergerste. Eleven new physiologic races have been identified and are numbered 12–22. Race 12 was found to be widely distributed in Great Britain, Portugal and Spain, the other ten were localized. Races 13, 14, 15 and probably 18 were found in Britain, races 18, 19 and 20 in Portugal, and races 16, 17, 21 and 22 in Spain.

3. A mutant, differing in colour (orange and yellow) and in pathogenicity, is recorded for *P. anomala*. The mutant arose from an unstable culture of race 14 cultivated on *Hordeum vulgare pallidum* (Sudan), and is numbered physiologic race 23.

4. Segregation and hybridization of physiologic races occurred through the aecidial stage on *Ornithogalum umbellatum* L. The heterozygous condition of physiologic races 12, 13 and 23 was demonstrated. Self-fertilized material from race 12 gave rise to the parent race, to races 16(?) and 19 and to four new races described under the numbers 24, 25, 26 and 27. From self-fertilized material of race 13, races 12(?), 13, 22 and 24

were isolated and also two new races numbered 28 and 29. From self-fertilized material of race 23, races 12, 19, 20 and 22 were isolated. A reciprocal cross between physiologic races 13 and 23 was achieved; in one direction the progeny gave rise to a single physiologic race, which proved to be new and is numbered 30; in the other direction the two physiologic races 30 and 18 were produced. This result is discussed and is attributed to cytoplasmic inheritance.

5. Several biotypes of physiologic races 12 and 13 were found; they seem to establish a linkage between the two races.

6. A new selection of differential hosts for the differentiation of physiologic races of *P. anomala* on cultivated barleys is proposed. A "Numerical Table" of the thirty physiologic races and an "Analytical Key" for their determination are given.

7. The distribution of physiologic races of *P. anomala* on cultivated barleys in Great Britain, Portugal and Spain is discussed.

8. From field observations it is concluded that at Cambridge *P. anomala* may overwinter in its uredospore stage. Evidence is given that in Portugal the uredospores survive the summer in the mountains.

REFERENCES

Brown, A. M. (1931). Investigations on the dwarf leaf rust of barley (*Pucc. anomala*). Report of Dominion Botanist for 1930. *Rep. Dep. Agric. Can.* p. 56.

Cotter, R. U. & Levine, M. N. (1932). Physiologic specialization in *Puccinia graminis secalis*. *J. agric. Res.* **45**, 297.

Craigie, J. H. (1927a): Experiments on sex in rust fungi. *Nature, Lond.*, **120**, 116.

—— (1927b). Discovery of the function of the pycnia of the rust fungi. *Nature, Lond.*, **120**, 765.

—— (1928). On the occurrence of pycnia and aecia in certain rust fungi. *Phytopathology*, **18**, 1005.

—— (1931). An experimental investigation of sex in the rust fungi. *Phytopathology*, **21**, 1001.

GASSNER, G. & STRAIB, W. (1932). Über Mutationen in einer biologischen Rasse von *Puccinia glumarum tritici* (Schmidt) Erik. und Henn. *Z. indukt. Abstamm.-u. VererbLehre*, **63**, 154.

GOLDSCHMIDT, W. (1928). Vererbungsversuche mit den biologischen Arten des Antherenbrandes (*Ustilago violacea* Pers.). *Z. Bot.* **21**, 1.

HEY, A. (1932). Beiträge zur Specialisierung des Gerstenzwergrostes, *Puccinia simplex* Erik. et Henn. *Arb. biol. Abt.* (*Anst.—Reichsanst.*), *Berl.*, **19**, 227.

HIRCHHORN, J. (1933). Dos royas de la cebada, neuvas para la Argentina. *Rev. Fac. Agron. La Plata*, **19**, 390.

JOHNSON, T., NEWTON, M. & BROWN, A. M. (1934). Further studies of the inheritance of spore colour and pathogenicity in crosses between physiologic forms of *Pucc. graminis tritici*. *Sci. Agric.* **14**, 360.

MAINS, E. B. (1917). The relation of some rusts to physiology of their hosts. *Amer. J. Bot.* **4**, 179.

—— (1926). Studies in rust resistance. *J. Hered.* **17**, 313.

—— (1930). Host specialization of barley leaf rust, *Pucc. anomala*. *Phytopathology*, **20**, 873.

MAINS, E. B. & JACKSON, H. S. (1926). Physiologic specialization in the leaf rust of wheat, *Pucc. triticina* Erik. *Phytopathology*, **16**, 89.

MAINS, E. B. & MARTINI, M. L. (1932). Susceptibility of barley to leaf rust (*Pucc. anomala*) and to powdery mildew (*Erysiphe graminis hordei*). *Tech. Bull. U.S. Dep. Agric.* no. 295.

MEHTA, K. C. (1933). Rust of wheat and barley in India. *Indian J. agric. Sci.* **3**, 939.

MURPHY, H. C. (1935). Physiologic specialization in *Puccinia coronata avenae*. *Tech. Bull. U.S. Dep. Agric.* no. 433.

NEWTON, M. & JOHNSON, T. (1932). Studies in cereal diseases. VIII. Specialization and hybridization of wheat stem rust, *Puccinia graminis tritici*, in Canada. *Bull. Dep. Agric. Can.* no. 160, N.S.

NEWTON, M., JOHNSON T. & BROWN, A. M. (1930a). A preliminary study on the hybridization of physiologic forms of *Puccinia graminis tritici*. *Sci. Agric.* **10**, 725.

—— —— (1930b). A study of the inheritance of spore colour and pathogenicity in crosses between physiologic forms of *Puccinia graminis tritici*. *Sci. Agric.* **10**, 775.

PELTIER, G. (1925). A study of the environmental conditions influencing the development of stem rust in the absence of an alternate host. III. Further studies of the viability of the uredospores of *Puccinia graminis tritici*. *Res. Bull. Neb. agric. Exp. Sta.* no. 34.

ROBERTS, F. M. (1936). The determination of physiologic forms of *Puccinia triticina* Erik. in England and Wales. *Ann. appl. Biol.* **23**, 271.

RONSDORF, L. (1934). Einige Versuche über biologische Rassen des Gerstenzwergrostes. *Arb. biol. Abt.* (*Anst.—Reichsanst.*), *Berl.*, **21**, 109.

—— (1935). Weitere Untersuchungen über den Nachweis biologischer Rassen des Gerstenzwergrostes, *Puccinia simplex* Erik. und Henn. *Phytopath. Z.* **7**, 236.

STAKMAN, E. C. (1930). Racial specialization of parasitic fungi. *Trans. Ky Acad. Sci.* **3**, 93.

STAKMAN, E. C. & LEVINE, M. N. (1922). The determination of biologic forms of *Puccinia graminis* on *Triticum* sp. *Tech. Bull. Minn. agric. Exp. Sta.* no. 8.

—— —— (1935). Analytical key for the identification of physiologic forms of *Puccinia graminis tritici*. From division of cereal crops and diseases. *U.S. Dep. Agric. Minn. agric. Exp. Sta.*

STAKMAN, E. C., LEVINE, M. N. & COTTER, R. U. (1930). Hybridization and mutation in *Puccinia graminis*. *Phytopathology*, **20**, 113.

Studies on Puccinia anomala *Rost.*

STAKMAN, E. C., LEVINE, M. N., CHRISTENSEN, J. J. & ISENBECK, J. J. (1935). Die Bestimmung physiologischer Rassen pflanzenpathogener Pilze. *Nova Acta Leop. Carol.* **13**, 319.

VAVILOV, N. J. (1919). Immunity of plants to infectious diseases. *Bull. Petrovsk. Acad.* (1918). (English résumé.)

WARD, H. M. (1902). On pure cultures of a Uredine, *Puccinia dispersa. Proc. roy. Soc.* **69**, 451.

—— (1903). Further observations on the brown rust of the Bromes (*Puccinia dispersa* Erik.) and its adaptive parasitism. *Ann. mycol., Berl.,* **1**, 132.

WATERHOUSE, W. L. (1927). Studies in the inheritance of resistance to leaf rust, *Puccinia anomala* Rost., in crosses of barley. *J. roy. Soc. N.S.W.* **61**, 218.

—— (1929). Australian rust studies. *Proc. Linn. Soc. N.S.W.* **54**, 615.

POSTSCRIPT. Further papers by W. STRAIB on *Puccinia anomala* (*Arb. biol. Abt.* (*Anst.—Reichsanst.*), *Berl.* (1937), **22**, 43 and *Züchter* (1937), **9**, 304) were received too late for consideration in connexion with my results.

B. D'OLIVEIRA.

CONIOPHORA PUTEANA (SCHUM.) KARST. ON LIVING *SEQUOIA GIGANTEA*

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(With Plate IV)

TREES of *Sequoia gigantea* growing in a garden in St Andrews were cut down in the spring of 1936. A piece of timber measuring approximately 30 in. in diameter and 12 in. in thickness from the base of a trunk was brought to the Botany Department. The annual rings exposed in cross-section showed the age of the tree to be 36 years. It was noted that there were considerable pockets of rotted wood in the outer portions at one side of the trunk, these being clearly visible on both transverse faces. Using some of this infected material as inoculum cultures were made in the manner described below. The rotted areas, which showed cubical cracking of the attacked wood, varied in size up to a maximum of approximately 5 in. by 2 in. The portion of trunk was left beside a potting shed till the autumn, in order to allow the wood to dry out. It was then planed smooth on the lower side and photographed to show the rotted portions (Pl. IV, fig. 1).

THE FUNGUS IN CULTURE

(*a*) *On agar*. The only organism isolated from the rotted areas was brought into culture on $2\frac{1}{2}$ % malt agar. Thin pieces of wood not exceeding 5 mm. in length were taken at random from the rotted areas. They were either washed with 1 % hydrochloric acid or dipped in methylated spirit and flamed before being placed on the surface of malt agar slopes contained in 6 in. test-tubes. After 7 days several of the cultures showed hyphae growing out from the inoculum on to the surface of the agar. The mycelia formed subsequently were all similar in appearance and continued to exhibit the same range of characters in all transfers.

Growth rate and type were studied on malt, prune and potato-dextrose agars. On the last-named medium the characters exhibited by the fungus were such that it fitted ínto Fritz's (1923) key for the identification of wood-destroying fungi along with the fungus causing Balsam rot type A. The principal characters used in making this determination

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were as follows. Growth at first white, silky, with prominent strands; becoming denser, woolly, with tangled, loose hyphae above the mat. Colour white, rapidly becoming tinged yellow; finally yellow more or less patched with brown, particularly where the medium tends to dry out. Slope covered in 10–14 days at 22° C. The hyphae bear single, paired and whorled clamp connexions. "Finger hyphae" were observed frequently, as figured by Kemper (1936). Fritz (1923) concluded that her cultures might be *Coniophora cerebella (puteana)*, but pointed out that they differed from earlier descriptions of that fungus in the absence of oidia (Möller, 1907). In the cultures obtained from *Sequoia* oidia were freely produced, and when old cultures were opened they were on occasion set free as visible white clouds. They appeared to be present in all cultures more than 14 days old. The growth rate of colonies in tube culture, on malt agar, was calculated over a period of 12 days. The figures obtained represented an average increase of 2·3 cm. in 2 days. This agrees well with the figure of 2·4 cm. given by Falck (1909) for *Coniophora cerebella (puteana)*. No oxidation rings were formed when the fungus was grown on a nutrient medium with additions of 0·5 % or 0·25 % tannic acid. This agrees with Bavendamm's (1936) findings for *C. cerebella (puteana)* and other fungi of his cellulose-attacking type. Attempts to induce the production of fructifications either on agar or wood blocks were unsuccessful. Nevertheless, the close agreement between the cultures derived from the infected *Sequoia* and cultures and descriptions of *C. cerebella (puteana)* leaves no doubt that they are the same.

(b) *On wood blocks.* Small blocks of various types of wood were placed on cotton-wool plugs in boiling tubes. The tubes were filled one-third full with water and then sterilized. Afterwards the wood blocks were inoculated on the upper end with mycelium from malt-agar culture. Blocks of sound *Sequoia* wood from both inner and outer regions of the trunk were employed. In addition the following woods were infected: lime, maple, horse-chestnut, hornbeam, alder, oak, elm, beech, willow and birch. The sequence corresponds with the declining order of the luxuriance of the mycelium produced on the respective woods. In all cases the growth was more vigorous than that produced on *Sequoia*. Characteristic mycelial strands were a prominent feature in the earlier stages of growth (Pl. IV, fig. 2).

Brown zone lines were formed on the surface of some of the wood blocks and against the glass of the containing tubes. In addition, irregularly shaped, dark brown structures appeared on the thicker mycelial threads. The largest of these reached a diameter of 2 mm. Many were not

visible individually with the naked eye, but were easily distinguished with a hand lens. They were composed of aggregates of thick-walled, brown pieces of hyphae and may be regarded as sclerotioid in nature.

The type of rot produced in culture is characterized by the appearance of horizontal cracks in the wood (Pl. IV, fig. 3). This is similar to the naturally produced rot. The loss in weight of artificially infected *Sequoia* blocks was estimated at approximately 43 % of the fresh weight after 9 months, and that of the control blocks at approximately 26 %, making the loss due to the activity of the fungus 17 % approximately.

The mycelium in both naturally and artificially infected wood was similar in appearance. It was readily picked out in sections stained by Cartwright's (1929) method. Hyphae were numerous, running along the wood elements and penetrating the lateral walls by means of the bordered pits and by bore holes. Clamp connexions and intercalary chlamydospores were observed.

PARASITISM OF THE FUNGUS

Fritz (1923) found a *Coniophora* associated with *Polyporus balsameus* in cubical rots of *Abies Balsamea* and *Picea mariana* in Canada. She was of the opinion that the former fungus was present as a secondary parasite. Meyer (1934) has recorded *Coniophora cerebella* on living birch near Moscow, maintaining itself in enclosed knots in the wood and subsequently rotting the felled timber. Robak (1936), examining brown cubical butt rots of spruce in Norway, isolated a *Coniophora* only and thought that it was of primary importance. In Britain, Day & Peace (1936), studying butt rot of conifers, state that *C. cerebella* (*puteana*), among other fungi, may be associated with this condition. European Larch and Norway Spruce are commonly affected and Scots Pine rarely; while Douglas Fir, Sitka Spruce, Japanese Larch, *Thuja* sp. and Lawson's Cypress have all been found seriously attacked.

As far as can be ascertained this is the first record of a *Coniophora* on *Sequoia gigantea*. In the present instance the fungus was the only organism isolated from the rotted areas of the trunk immediately after felling. In these circumstances it seems clear that it was acting as a primary parasite.

The increasing number of records of *Coniophora puteana* on living wood serves to draw attention to an important source of infection of timbers which may be used in constructional work.

SUMMARY

1. When a 36-year-old tree of *Sequoia gigantea* was felled it was found to have rotted areas in the wood at the base of the trunk.

2. One organism only was isolated from wood in these rotted areas.

3. The general and detailed microscopic characters exhibited by this fungus in culture identify it as *Coniophora puteana*.

4. In the present case it seems clear that the fungus was acting as a primary parasite.

REFERENCES

BAVENDAMM, W. (1936). Erkennen, Nachweis und Kultur der holzverfärbenden und holzzersetzenden Pilze. *Hand. biol. ArbMeth.* Abt. **12**, 927.

CARTWRIGHT, K. ST G. (1929). A satisfactory method of staining fungal mycelium in wood sections. *Ann. Bot., Lond.*, **43**, 412.

DAY, W. R. & PEACE, T. R. (1936). Butt rot of conifers. *Scot. For. J.* **50**, 52.

FALCK, R. (1909). Die Lenzitesfäule des Coniferenholzes. *Hausschwammforsch.* **3**, 1.

FRITZ, C. W. (1923). Cultural criteria for the distinction of wood-destroying fungi. *Trans. roy. Soc. Can.* **17**, sect. v, 191.

KEMPER, W. (1936). Zur Morphologie und Zytologie der Gattung *Coniophora* (*usw.*) insbesondere des sogenannten Kellerschwamms. *Zbl. Bakt.* Abt. II, **97**, 100.

MEYER, E. I. (1934). Black knots and darkening of the heartwood in Birch in relation to the initiation of timber rotting. *Rev. appl. Mycol.* **14**, 269.

MÖLLER, A. (1907). Hausschwammuntersuchungen. *Hausschwammforsch.* **1**, 29.

ROBAK, H. (1936). Notes on Norwegian wood rots. II. The genus *Coniophora* DC. and the " *Vaporarius* rot" in conifers. *Nyt. Mag. Naturw.* **76**, 2.

EXPLANATION OF PLATE IV

Fig. 1. Cross-section of *Sequoia* trunk showing dark brown rotted areas towards the foot of the photograph.

Fig. 2. Birch block artificially infected with *Coniophora puteana*. Characteristic strands have formed on the surface of the bark.

Fig. 3. *Sequoia* wood block, artificially infected with *Coniophora puteana*, nine months after infection. Horizontal cracking of the wood can be seen.

Fig. 1.

Fig. 2.

Fig. 3.

MACDONALD.—*Coniophora puteana* (Schum.) Karst. on Living *Sequoia gigantea*
(pp. 83–86)
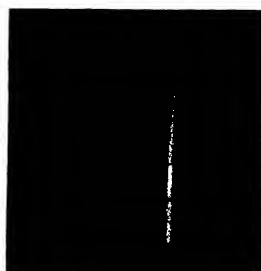
NOTES ON THE PHOTOPERIODIC REACTIONS AND VIRUS CONTENTS OF SOME PERUVIAN POTATOES

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(With Plates V and VI)

CONTENTS

I. INTRODUCTION

IN December 1937 a consignment of fifty-nine potato varieties cultivated at Puño and collected there by the Percy Sladen Expedition to Lake Titicaca was received at the Potato Virus Research Station, Cambridge. Only the vernacular names of the varieties were supplied by the collectors and their botanical relationships have not been fully worked out. Two forms of Papa surimana, with pink and purple tubers respectively, appear to belong to *Solanum chaucha*; Azul parcco, Parcco caramo, Parcco hancco, Luqui mari and perhaps Pocco tturo huilla appear to form a homogeneous group so far unidentified, and most of the other varieties may provisionally be regarded as forms of *S. andigenum*.

The tubers were grown in an insect-proof greenhouse with a view to their multiplication and distribution as an adjunct to the Empire Potato Collection now being made by an expedition to Mexico and South America. During 1938 investigations were carried out to determine their photoperiodic reaction and their approximate virus content.

II. INFLUENCE OF LENGTH OF DAY ON GROWTH AND YIELD

The influence of length of day on the growth of the domestic potato has been studied by Garner & Allard (1923) and Tincker (1925). The former found that in the case of the McCormick variety an increase in the period of illumination from the normal summer day (14–15 hr.) to 18 hr.

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completely inhibited tuber formation, while, although flower buds were formed, they did not open. When the length of day was artificially restricted the plants bloomed earlier than the controls, and with periods of 10 and 13 hr. illumination the ratio of tubers to top was greater than with the controls. With the 10 hr. day this ratio was at a maximum, but the actual yield per plant was less than in full daylight. These workers similarly found that tropical yams (*Dioscorea alata*) gave a higher yield of tubers with 10 or 12 hr. illumination than with full summer day, and that Peruvian and Bolivian varieties of bean (*Phaseolus vulgaris*) only flowered under conditions of 12 hr. days or less (Garner & Allard, 1920). Tincker, working with King Edward potato, found the optimum length of day for tuber formation to be 12 hr. Yields of plants receiving only 9 hr. light were greatly inferior to those from full summer day controls. The collection of potato varieties used in the present work was obtained from a locality in latitude 15° 53′ S., i.e. one where the length of day throughout the year varies approximately between 11 and 13 hr. Russian workers have shown that South American potato varieties collected from within the tropics do not yield well under the long-day conditions obtaining during summer at Leningrad, and that with them the optimum length of day for tuber formation is 9 hr.

The first crop of Peruvian potatoes raised at Cambridge was planted in December 1937 and harvested before the end of April 1938; thus growth was nearly completed before long-day conditions set in. A second group of tubers was potted in duplicate at the beginning of March. One set was given a 9 hr. day while the other was exposed to the full length of day experienced during summer at Cambridge (52° 12′ N.), varying between about 13 hr. at the beginning of April and over 16 hr. in mid-June. The experiment was intended merely to test the current view that Peruvian potatoes would not form tubers under long-day conditions, and estimation of yield was not contemplated. As only a small dark chamber was available the short-day plants were packed close together in 7 in. pots. The long-day plants were better spaced and had 8 in. pots and, contrary to expectation, most of them produced tubers. Direct comparison between yield obtained in the two sets is unprofitable, but in those cases where in spite of their more favourable surroundings the long-day plants yielded noticeably fewer tubers than the corresponding short-day ones, one seems justified in accepting the influence of length of day to explain the discrepancy.

The effect of long-day conditions on these "short-day" plants may be summarized as follows:

(1) *Effect on flowering.* Of the long-day series thirty-five out of fifty-six flowered; of the short-day group only two varieties flowered, viz. Lanta mari and Hanco cuillo, and these did so about a fortnight later than the corresponding plants in the other set.

(2) *Effect on time of maturity.* As shown in Table I, the short-day plants matured over a period of about 1 month, after growing periods of 15–19 weeks. The long-day series, on the other hand, matured fairly uniformly during the second and third weeks of July.

(3) *Effect on tuber formation.* The effect on tuber formation involves two considerations, yield and dormancy. In Table I the varieties are classified under three main heads:

A. Those which produced good crops of normal tubers under long-day conditions.

B. Those which under the same conditions produced good crops in which the dormancy period was eliminated. This forms by far the largest group and may be subdivided into: (1) Varieties in which second growth occurred but in which the tubers did not send sprouts above ground. (2) Varieties in which the tubers produced long sprouts which came above ground to form erect green shoots, clustered round the base of the parent plant before the latter matured (Pl. V, fig. 4). The most extreme case in this group was Chachaza which formed eight primary tubers, each of which gave rise to a young plant bearing secondary tubers and stout aerial shoots. In other varieties tubers were produced by lateral buds, while the apex of the stolon grew for a long distance and finally turned up to form a leafy shoot. (3) Varieties in which the crop, though of fair quality, was less than that produced by the corresponding 9 hr. day plant.

C. Varieties in which tuber production was negligible under long-day conditions. Most of the plants produced a mass of long stolons which formed a felted mass round the edge of the pot and whose tips finally turned above ground as green shoots (group C (1)). Garner & Allard record production of somewhat similar offsets in McCormick potato subjected to 18 hr. illumination. In a small group (C (2)) consisting mainly of the "Parcco" varieties, stolon formation was almost inhibited. Oca (*Oxalis tuberosa*) and Papa lisa (*Ullucus tuberosus*) also fall in group C as regards tuber production.

It will be obvious that the groups outlined above are not to be regarded as hard-and-fast divisions. Thus, B (3) forms a transition zone between B and C, and it is possible that further experience may remove some of the varieties from group A to group B.

Table I. *Influence of length of day on tuber formation of Peruvian potatoes potted 2 March* 1938

Variety	Date of harvest	9 hr. day			Full day		
		No. of tubers	Greatest length of tubers in.	Wt. of tubers g.	No. of tubers	Greatest length of tubers in.	Wt. of tubers g.
Group A. Varieties yielding good crops of normal tubers under long-day conditions							
Alpaca noza	13. vii	10	1⅜	61	14	1⅞	208
Lanta mari	2. vii	10	1	32	32	1⅜	170
Cochicallo	15. vi	45	1⅝	91	20	2	94
						Average	157
Group B. Varieties yielding good crops of abnormal tubers under long-day conditions							
(1) Much second growth but no sprouting observed							
Papa ppitiquiña	15. vi	11	⅝	12	34	1¼	143
Condor huallcco	13. vii	16	1¼	114	13	2¼	158
Quero chiquilla	15. vi	23	⅞	49	28	2¼	120
Pichuya choque	15. vi	20	1⅜	80	31	2	187
Hanco cuillo	13. vii	22	1¼	90	23	1½	152
Ppicochara	15. vi	22	1¾	63	16	2	87
						Average	141
(2) Good crops of tubers which produced aerial shoots before harvest							
Papa (black) surimana	13. vii	14	1	14	17	1⅞	87
Papa (red) surimana	2. vii	24	1⅜	60	39	1⅜	193
Azul surimana	15. vi	22	1¼	66	21	1⅞	126
Papa alcayurima	13. vii	31	¾	68	19	1½	140
Papa negra yanahimilla		11	1¼	56	16	2	197
Panti imilla		11	1½	60	17	1¾	191
Pinta milagro	2. vii	23	1	60	25	1¾	216
Chuhuavisca	13. vii	12	1¾	62	46	1½	190
Puca ppitiquiña	13. vii	26	1¼	110	25	1½	208
Amajaya arama	15. vi	20	1¼	93	32	1½	220
Yana imilla	2. vii	17	1	80	13	1½	105
Chupica imilla	2. vii	35	1	83	13	1¼	83
No. 162 Granja Salcedo	16. vi	22	1½	62	14	2	144
Chachaza	15. vi	14	1½	60	8 + 7	1½	93
Sacampai choque	15. vi	27	2¼	64	19	1½	98
Huilla mari	13. vii	8	1⅜	88	13	1⅜	136
Chohuahineca	13. vii	19	1¼	77	37	2	157
Ppujno	13. vii	12	1¾	106	14	1¾	139
Cacho amajaya	15. vi	32	1¼	84	22	2¼	150
Huaca nuño	13. vii	33	1⅜	100	16	3	123
Chupica yarama	13. vii	15	1¼	92	15	2¼	155
Huilla imilla	15. vi	23	1	81	38	1⅜	162
Alcca fina	13. vii	26	2¼	116	25	2	221
Huaca huajra	13. vii	26	1¾	103	17	2¼	136
Ccompis	16. vi	14	1¼	101	21	1¼	138
Chiquiña	15. vi	13	1⅝	61	9	1¼	69
Ruqqui	13. vii	14	2	100	16	1¼	155
Huaña	16. vi	16	1¼	88	13	2¼	224
Sihuanco choque	15. vi	22	1¼	65	23	1¾	113
Quello huaccoto	13. vii	45	1¼	127	11	1⅜	160
Futaco	13. vii	21	1¼	109	11	1¼	139
Ofrenta	16. vi	14	1⅜	96	25	2	233
						Average	154

Table I (cont.)

Variety	Date of harvest	9 hr. day			Full day		
		No. of tubers	Greatest length of tubers in.	Wt. of tubers g.	No. of tubers	Greatest length of tubers in.	Wt. of tubers g.
B (3) Poor crops of tubers which have produced aerial shoots							
Papa runtusa	16. vi	33	1	87	14	1¼	90
Phospa sunchus	16. vi	15	1½	91	6	1¾	44
Liquelique ppiqhe	15. vi	32	1	86	16	1¼	68
Ccosi	15. vi	20	2	103	16	1¾	95
						Average	74
Group C. Yield negligible or nil under long-day conditions							
(1) Stolons produced							
Papa ruqui	12. v	16	½	15	3	¾	3
Papa negra	12. v,	35	¾	21	9	¾	5
Chinamajaya	15. vi	29	1¼	64	1	1	8
Luqui mari	2. vii	10	1¼	64	3	1	10
Milagro	15. vi	32	1¼	63	1	1¼	8
Azul lajra	13. vii	19	1½	82	0	0	0
Poccoye	2. vii	34	1¼	61	0	0	0
						Average	5
(2) No stolons produced (one short stolon in Azul parcco)							
Pocco tturo huilla	13. vii	5	1¼	28	0	0	0
Parcco hancco	13. vii	14	2¼	86	0	0	0
Parcco caramo	13. vii	13	1¼	60	0	0	0
Azul parcco	13. vii	25	2	90	1	¾	5
						Average	1¼

From these data it seems that the varieties of groups A, B (1) and possibly B (2) could be grown in England with a prospect of fair tuber production, especially if they were planted early. Possibly had the tubers been planted 1 or 2 months later the adverse effect of long-day conditions might have been more marked. It was partly on a basis of their short-day habit that Russian authors considered the Peruvian potatoes unlikely ancestors of the domestic *S. tuberosum*, but it appears that this objection does not apply to all the varieties grown at Puño.

III. VIRUS CONTENT

The Peruvian potato varieties were subjected to the routine examination pursued with all new varieties received at the virus station. This involves sap inoculation to *Nicotiana tabacum* (variety White Burley), *Datura stramonium* and *Capsicum annuum* (variety Golden Dawn), and the grafting of unhealthy looking stocks to standard potato varieties such as Epicure, President, Arran Victory and Up-to-Date. The sap inoculations are sufficient to detect viruses Y, X, F and G, while suitable grafts reveal the presence of viruses B, C, A and leaf roll. In order to check the

presence of very weak strains of X, which may be carried by tobacco or *Datura* without evoking a mottle, it is customary to reinoculate such hosts with a severe "ring-spot" type of X. If infection does not follow reinoculation the presence of a weak or masked strain of X may be inferred (Salaman, 1933). It was not practicable to carry out a complete investigation of all the fifty-nine varieties, but all were tested by sap inoculation to tobacco, *Datura* and *Capsicum*, and the majority were also grafted to healthy President potato. Varieties whose virus content appeared of special interest were chosen for further investigation. The results may be summarized as follows:

(1) *Healthy varieties.* No sap-transmissible virus was found in the varieties Ccompis, Ccosi, Chinamajaya, Milagro, Papa negra, Phospa sunchus, Piñazo, Poccoye, Ppicochara, Quero chiquilla and Sacampai choque, all of which appeared healthy under glasshouse conditions.

(2) *Virus X.* The presence of a virus resembling X was detected in twenty-eight varieties. In fifteen of these it appeared to be the only virus present, in nine it occurred in association with B and in two with a streak virus similar to C. In Alcca fina X was associated with a virus of the Aucuba group. In Chiquiña X was associated with a virus which killed Di Vernon by top necrosis. Of the varieties containing X alone, Chupica imilla, Granja Salcedo no. 162, Hanco cuillo, Huaña, Huilla mari, Liquelique ppiqhe, Papa ppitiquiña, Papa surimana (purple-tubered form) and Pichuya choque appeared healthy under greenhouse conditions, whilst Alpaca noza, Azul surimana, Huilla imilla, Ofrenta, Puca ppitiquiña and Ruqqui exhibited a mild interveinal mottle, most pronounced in the last named. The strains of X recovered were mostly of a mild type, giving rise to interveinal mottles on tobacco and *Datura* with no local lesions. Chiquiña, Granja Salcedo no. 162, Papa surimana and Ruqqui contained strains so weak as to evoke no symptoms on the above-mentioned plants, though capable of protecting them against reinfection with a stronger strain and of killing Epicure and Arran Crest by top necrosis. The Alcca fina and Huilla imilla strains induced faint ring-like local lesions in *Datura* (Pl. VI, fig. 5), whilst the form obtained from Pichuya choque and Azul surimana produced a few scattered yellow spots superimposed on the general mottle in tobacco. Attempts to isolate a ring-spot strain by punching out these yellow spots and inoculating them to fresh plants failed.

Papa ppitiquiña yielded an X which resembled Bawden's strain D (Bawden, 1934). On tobacco and *Datura* it gave rise to mild interveinal mottles only; on *Capsicum* grey local lesions appeared, followed by

systemic interveinal necrosis and die-back. President and Arran Victory infected by graft or by sap developed a blotchy interveinal mottle with large rounded grey-brown interveinal necroses and some leaf drop. King Edward was killed by top necrosis. A somewhat similar strain, inducing less severe foliar necroses on President, and interveinal mottle only on Arran Victory, was recovered from Puca ppitiquiña. The virus of Alpaca noza gave rise to a rather bright interveinal mottle on President (Pl. V, fig. 1).

(3) *The Aucuba group*. Viruses inducing host reactions which recall those of the Aucuba or yellow mosaics of the potato (viruses F and G) were found in Alcca fina, Chachaza, Huaca huajra, Luqui mari, Panti imilla, Papa ruqui, Parcco caramo, Pocco tturo huilla, Ppujno, Puca muru chimaco and Sihuanco choque. The general characters on which attribution to this group is based are: absence of symptoms on tobacco and *Datura* associated with failure to protect these plants against reinoculation with severe X, local lesions and systemic interveinal necrosis, sometimes followed by leaf drop and even die-back on *Capsicum*, and failure to induce top necrosis in X-intolerant potato varieties such as Epicure and King Edward. In the case of Alcca fina, where a strain of X was also present, the occurrence of an Aucuba-type virus was inferred mainly from the reaction of Dunbar Yeoman. Symptoms in the original potatoes varied from none in the case of Huaca huajra, Panti imilla and Parcco caramo to more or less bright interveinal mottle in Alcca fina, Chachaza, Luqui mari, Papa ruqui, Ppujno, Pocco tturo huilla and Puca muru chimaco. In the case of the third and sixth named the mottle was followed by roundish black interveinal blotches with irregular zoning of the target-blotch type, leading ultimately to a dropping of the older leaves (Pl. VI, fig. 6). No fungus could be found in the foliar lesions nor could the symptoms be transmitted by grafting to standard British domestic potato varieties. Similar necrotic spots appear on the leaves of certain seedlings raised from the domestic potato and appear to bear no relation to their virus content. In Sihuanco choque a bright Aucuba-like mottle was observed.

Some difficulty is met with in the attempt to group these viruses under the two individuals F and G recognized by Clinch et al. (1936). Those from Panti imilla and Huaca huajra were carried without symptoms by Epicure, but the former gave rise to grey marginal necrosis on the lower leaves of Dunbar Yeoman and is therefore perhaps not a true Aucuba (G). The virus of Sihuanco choque, which induces an Aucuba mottle on its original host, and of Papa ruqui which was carried by Dunbar Yeoman

and induced mottlings and ruffling in King Edward, may be true G. The viruses of Alcca fina, Chachaza, Luqui mari, Pocco tturo huilla, Ppujno and Puca muru chimaco all induced marginal and terminal necrosis or scorching in Dunbar Yeoman leaflets and hence resembled F or tuber-blotch virus. A virus apparently identical with tuber blotch occurred associated with a streak in Parcco hancco (see §5(c)). Tuber symptoms were observed only with Puca muru chimaco virus on President, where faint internal brown spots were seen in one tuber out of four. It is possible that the examination of the tubers, made at the time of harvest, was premature, and that further symptoms of tuber blotch would have developed in them during storage.

(4) *Virus B*. The following potato varieties contained, in addition to X, a virus graft-transmissible to President, in which it caused top necrosis: Azul lajra, Cacho amajaya (Plant 1), Condor huallcco, Futaco, Huaca nuño, Papa alcayurima, Papa negra yanahimilla, Papa surimana (pink-tubered form) and Yana imilla. A similar virus, apparently free from X occurred in Azul parcco and Quello huaccoto. All these varieties appeared healthy with the exception of Azul lajra, Cacho amajaya and Futaco which showed a mild interveinal mottle. In the case of Papa alcayurima and Papa negra yanahimilla the virus also induced top necrosis in Arran Victory and was therefore probably Up-to-Date streak or virus B. The same conclusion may be reached for the Condor huallcco virus, which was carried by the U.S.D.A. seedling 41956, a variety usually killed by the other known streak, virus C. A second plant of Cacho amajaya, derived from a different tuber in the original consignment, appeared free from all viruses, as inferred from the results of grafts to Epicure and President.

(5) *Virus C*. Di Vernon streak or C has not been very fully studied, but the viruses present in the following varieties appear to resemble it, having distinctive characters in common with it:

(a) Amajaya arama and Pinta milagro. Both these varieties contained a mild X together with a virus which caused top necrosis on President but had no visible effect on Arran Victory.

(b) Huaca lajra was apparently free from X, Y, F or G since no symptoms resulted from inoculation to tobacco, *Datura* or *Capsicum*, but when grafted to President, Arran Victory, British Queen, Epicure and Majestic the stocks died of top necrosis. These reactions may be tentatively ascribed to the presence of both streak viruses, B and C, since although B can infect tobacco, *Datura* and *Capsicum*, it is not always readily sap-transmissible from potato to these hosts.

(c) Parcco hancco, which showed a rather fleeting veinal mottle of some upper leaves but had no other symptom of disease, contained a virus of the Aucuba group plus a streak virus similar to C. Sap inoculations to tobacco and *Datura* evoked no symptoms in the hosts nor were the tobaccos protected against reinoculation with severe X. Sap inoculation to *Capsicum* induced local lesions, systemic interveinal necrosis and leaf drop, indicating the presence of virus F or G. When grafted to healthy plants of U.S.D.A. seedling 41956 (two plants), President, Up-to-Date, Katahdin and Sharpe's Express, and to a President previously infected with a mild X, top necrosis resulted in the stock (Pl. V, fig. 2). Attempts to transmit the top necrosis virus by sap inoculation from the dying U.S.D.A. 41956 to healthy U.S.D.A. 41956, President, Arran Victory and Katahdin gave negative results. When two plants of Arran Victory were grafted from Parcco hancco their young leaves became slightly ruffled with interveinal mottle, while the intermediate and lower leaves exhibited numerous small interveinal necrotic spots and rings (Pl. V, fig. 3). The plants were not killed and the tubers showed no sign of necrosis. On Dunbar Yeoman there was no top·necrosis but large interveinal necroses developed on the intermediate leaves, followed by general chlorosis and leaf drop. Streaks appeared on the stem and petioles but the growing point remained healthy.

On Epicure an interveinal mottle with light superficial necrosis of the upper leaves developed about a fortnight after grafting. Three weeks later all except the uppermost leaves were chlorotic and beginning to drop. After a further 10 days the shoots were all nearly dead, all the leaves were shrivelled and hanging, though still firmly attached to the stem. The tips had wilted but were not blackened as in typical top necrosis. These symptoms resemble strikingly those of tuber blotch on this variety as described by Clinch *et al.* (1936). In the case of Dunbar Cavalier the shoot next the Parcco hancco scion died of top necrosis, whilst two other shoots showed leaf drop, crinkling and light superficial foliar necroses only.

(6) *Possible new viruses.* In six Peruvian potato varieties, viruses or virus complexes were discovered whose reactions could not be readily interpreted in terms of the known potato viruses. The varieties were:

(a) *Papa runtusa.* This plant appeared healthy apart from a faint interveinal mottle on a few intermediate leaves. Sap inoculations from it to tobacco, tomato and *Lycium bàrbarum* led to no visible reactions, but the first-named plants were found to be protected against reinoculation with severe X. Sap inoculation to *Datura* did not result in local lesion formation, and for 16 days the inoculated plants remained healthy.

Some days later, however, the young leaves became deformed, with marked rugosity, faint brownish necrosis of the veins and a downward curling of the lamina (Pl. V, fig. 5). Five weeks after inoculation the leaves were waved, with a faint bright yellow veinal mottle and a few brownish necrotic specks on the finer veins. On *Capsicum annuum* brown local lesions were followed by systemic interveinal necroses.

Grafts from the original potato to Epicure (three plants) resulted in no well-defined disease, although the leaves of the stock were unusually dark green and glossy with slight waving. The scions grew well and formed good union with the stocks, while the presence of virus in the latter was demonstrated by grafting from them to healthy plants of President potato, which rapidly succumbed to top necrosis. Dunbar Yeoman similarly grafted developed large marginal necroses on the lower leaves, followed by general chlorosis and leaf drop. Grafted plants of Arran Victory, U.S.D.A. 41956 and Eclipse appeared unaffected by the viruses present. Reviewing the above data we might infer the presence of *F* or a related virus, from the Dunbar Yeoman and *Capsicum* reactions. The virus causing top necrosis of President cannot, however, be identified with either of the streak viruses known in this country, since *C* would have killed Eclipse and probably U.S.D.A. 41956, and *B* kills Arran Victory and Epicure.

(*b*) What seems a similar virus occurred in Chuhuavisca. Here again sap inoculation to tobacco produced no visible result at first, but the plants were protected against reinoculation with severe *X*. Over a month after infection, however, a few yellow rings appeared on the reinoculated and non-inoculated plants alike. On *Datura* there was a whitish semi-necrotic etching of the veins, first observed 16 days after inoculation, but the systemic veinal mottle described under 6(*a*) did not persist. Inoculated *Capsicum* plants exhibited local lesions followed by severe systemic necrosis. Epicure (two plants) was unaffected by grafts from Chuhuavisca, though the scions made good unions and grew vigorously to a height of 2 ft. Grafts from the original potato to President resulted in a spotty mottle on the stock with scattered superficial necroses such as is sometimes seen with Up-to-Date streak on this host and may best be regarded as an arrested form of top necrosis. U.S.D.A. 41956 remained apparently healthy when grafted with Chuhuavisca.

Both the above viruses are clearly related to the *X* group, as shown by their complete protection against reinoculation with severe *X*, but they differ from all known strains of that virus in failing to cause top necrosis in Epicure.

(c) *Chohuahineca*. This plant, which showed no sign of disease, contained a virus differing from all the potato viruses known in this country in being readily sap-transmissible to *Datura* and not to tobacco. Tobacco plants inoculated from Chohuahineca remained perfectly healthy, nor could any virus be recovered from them causing symptoms on *Datura*.

Direct sap inoculation from Chohuahineca to *Datura* resulted in formation of a few round brown local lesions 2–3 mm. across, first observed 12 days after inoculation (Pl. VI, fig. 3). Systemic symptoms were not seen until 1 month after inoculation, when a mild systemic interveinal mottle developed, which became brighter yellow-green with a few yellow spots but was not associated with ruffling or deformity of the leaves. When the local lesions were punched out and inoculated to young *Datura* plants fresh local lesions resulted, followed by much more severe interveinal mottle with small necroses, deformity of the leaves and stunting of the plants (Pl. VI, fig. 4). It seems, therefore, that a mixture of virus strains was present and that the most severe as regards *Datura* could be selected by inoculating only from the local lesions. Inoculation to *Nicotiana glutinosa* induced a mild systemic interveinal mottle with no local lesions. No symptoms resulted from inoculation to tomato, nor could the virus be recovered from this host. Inoculation to *Capsicum* gave no local lesions but a mild systemic interveinal mottle with small interveinal necroses. Inoculation to *Lycium barbarum* led to negative results or to the formation of faint local rings only.

Grafting of Chohuahineca to Epicure induced no disease in the stock, while in a similarly treated King Edward only a bright interveinal mottle was produced, with some rugosity and waving of the leaves (Pl. VI, fig. 2). From Epicure the virus was recovered unchanged on *Datura*. Scions from a second plant of Chohuahineca killed Epicure, President, President carrying mild X, Sharpe's Express and British Queen with top necrosis. This plant probably therefore contained a strain of X as well as the streak virus. Grafted plants of Arran Victory and Up-to-Date remained healthy, although the presence of the virus in the former was confirmed by grafting back to President which then developed top necrosis. Similar grafts from Chohuahineca resulted in blotchy veinal mottle with slight welling and waving of the leaves on Katahdin (Pl. VI, fig. 1) and in veinal necrosis followed by severe chlorosis and leaf drop on Eclipse. A grafted plant of U.S.D.A. 41956 developed a bright veinal mottle with no necrosis but a dropping of the lower leaves. Grafts from this plant to President led to top necrosis of the latter, but neither local nor systemic symptoms resulted from sap inoculations to Arran Crest, Arran Pilot, Arran

Victory, Doon Early, Doon Star, Dunbar Yeoman, Katahdin, President, Up-to-Date or U.S.D.A. 41956. Inoculation from the same plant to *Datura*, however, gave local lesions and systemic interveinal mottle as described above, while no reaction followed inoculation to *Capsicum*. This *X*-free stock of the Chohuahineca virus was used in a study of its physical properties in *Datura* sap with the following results. The virus was recovered unchanged after heating for 10 min. at 60° C. but not at 65° C. It was recovered on six out of six *Datura* plants after dilution to 1 in 10,000, on two out of six after dilution to 1 in 100,000 and on none out of six after dilution to 1 in 1,000,000. It survived at least 5 days ageing *in vitro*.

This virus bears considerable resemblance to Up-to-Date streak, virus *B*, but differs from it in not infecting tobacco and tomato and in its effect on certain potato varieties. Its reactions are compared with those of *B* and *C* in Table II where + indicates top necrosis (cf. Bawden, 1936).

Table II. *Reactions of Chohuahineca virus, and viruses B*
and C on certain potato varieties

	Epicure	Presi-dent	Arran Victory	Up-to-Date	British Queen	Katah-din	U.S.D.A. 41956
Chohuahineca	–	+	–	–	+	–	–
B	+	+	+	–	+	+	–
C	?	+	–	+	+	?	+

The variety Chupica yarama apparently contains a similar virus.

(*d*) Two other varieties, Cochicallo and Lanta mari, both infected with a mild *X*, also contained streak viruses which were not fully investigated. That of the former killed Arran Victory with top necrosis, induced bright yellow interveinal mottle on British Queen, and gave no visible reaction on U.S.D.A. 41956, Epicure, President or Up-to-Date. That of the latter killed Dunbar Yeoman with top necrosis but had no visible effect on President.

(7) *Leaf roll.* None of the Peruvian varieties showed typical symptoms of leaf roll early in the season, but a number exhibited a rolling of the lower and intermediate leaves as they approached maturity. These were Azul parcco, Hanco cuillo, Huaca huajra and Parcco caramo. In the case of Huaca huajra the symptoms of leaf roll developed within 10 weeks after emergence of the plant above ground. It was then 30 in. high with the lower leaves harsh in texture, much rolled with interveinal pallor and some anthocyanin development on the lower surfaces. Grafts to British Queen, Majestic and President failed to transmit leaf roll as the stocks

died of top necrosis, but in the case of a graft to Epicure one shoot of the stock died of top necrosis while two others developed symptoms resembling leaf roll.

A graft from Parcco caramo to President induced slight interveinal pallor and very slight anthocyanin development on the lower leaves, but full symptoms of leaf roll did not appear.

In the case of Yana imilla one plant which exhibited a bright yellow Aucuba-like mottle on most leaves apparently contained viruses $X + B$ (see (4), p. 94). Another plant, which appeared healthy, when grafted to President induced typical symptoms of leaf roll on some lower leaves; these were followed by a long growth of healthy shoots, until some 4 months after grafting the upper leaves developed interveinal pallor in patches towards the margin with traces of anthocyanin on the lower surface. Rolling of leaves also resulted in a plant of Dunbar Cavalier grafted from the same source.

Chachaza, which only showed slight interveinal mottle and no symptoms of leaf roll, also induced interveinal pallor, rolling, pointing and anthocyanin development in a plant of President potato, some 5 weeks after grafting. Later, however, the new growth of stock developed in a normal manner, though the lower leaves remained rolled.

The evidence of presence of leaf roll is thus not conclusive but it seems likely that strains of this or a similar virus occurred in the varieties mentioned above.

(8) *Viruses A and Y.* No evidence has been obtained of the presence of either of these viruses. All plants whose appearance at all suggested crinkle were grafted to Up-to-Date or British Queen, but the stocks either remained healthy or, if top necrosis supervened, there was clear evidence from other sources of the presence of a streak virus capable of bringing about such a reaction.

All plants suspected of infection with Y, in addition to the routine test on tobacco, which should have detected that virus, were inoculated to seedlings of *Lycium barbarum*, lately shown to be a useful diagnostic host for Y (Dennis, 1938).

IV. Infection of healthy Peruvian potatoes with European viruses

A small amount of material of the varieties listed in (1), p. 92, was available for infection experiments designed to explore their reactions to European viruses. The results may be summarized briefly as follows:

(1) *Ccompis.* Developed typical leaf roll when grafted from infected British Queen. Developed interveinal mottle when grafted with President carrying a mild *X*.

(2) *Ccosi.* Developed typical leaf roll when grafted from infected British Queen. Carried *X* when grafted with that virus in Great Scot. The virus was recovered from Ccosi by inoculation to *Datura*.

(3) *Milagro.* Succumbed to top necrosis when grafted with Di Vernon carrying *X + C*. Remained healthy when sap-inoculated with mild *X* alone.

(4) *Phospa sunchus.* Developed typical leaf roll when grafted with infected British Queen. Remained apparently healthy when grafted with Di Vernon carrying *X + C*, but scions taken from it to President killed the stock with top necrosis. Developed typical veinal mottle, rugosity and leaf drop when grafted with *Y·* in Bintje.

(5) *Sacampai choque.* Succumbed to top necrosis when inoculated with mild *X*. Developed only veinal mottle with neither necrosis nor leaf drop when grafted with *Y* in Bintje.

Tubers of a small number of varieties, which remained after all other requirements had been met, were planted in the field and exposed to natural infection by aphids. Under these conditions plants of the following varieties developed symptoms of leaf-drop streak: Azul parcco, Cacho amajaya, Ccosi, Chinamajaya, Huaca huajra, Huaca nuño, Phospa sunchus, Pinta milagro.

V. Summary

Exposure of the Peruvian potato varieties to full summer-day conditions at Cambridge resulted in a more uniform growing period, and increased the tendency to bloom. The effect on tuber production varied according to the variety. In most types the weight and number of tubers were not adversely influenced, but there was a tendency to elimination of the dormancy period and to conversion of the stolons into aerial shoots. In a few varieties there was no adverse influence, and in the members of one small group plants exposed to full-day conditions formed no tubers.

Investigation of virus content showed that of fifty-nine varieties only eleven were healthy. In the remaining forty-eight varieties there were found viruses apparently identical with those known in this country as *X, B, C, F, G* and perhaps leaf roll.[1] There was also evidence of the presence of other viruses, suggesting the existence in South America of a virus

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[1] Equivalent respectively to the *Solanum* viruses 1, 4, 5, 8, 9 and 14 of Dr K. M. Smith's classification. *Solanum* virus 6 of the same classification is the foliar necrosis strain of *X* (see p. 92).

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complex, only isolated members of which have hitherto been known in Europe. It follows that great caution should be exercised in introducing South American varieties into potato-growing districts.

The writer's thanks are due to Dr R. N. Salaman who suggested and provided facilities for carrying out the scheme of work.

REFERENCES

BAWDEN, F. C. (1934). Studies on a virus causing foliar necrosis of the potato. *Proc. roy. Soc.* B, **116**, 375–95.

—— (1936). The viruses causing top necrosis (acronecrosis) of the potato. *Ann. appl. Biol.* **23**, 487–97.

BUKASOV, S. M. (1933). *The Potatoes of South America and their Breeding Possibilities.* Leningrad.

CLINCH, P. E. M., LOUGHNANE, J. B. & MURPHY, P. A. (1936). A study of the aucuba or yellow mosaics of the potato. *Sci. Proc. R. Dublin Soc.* N.S. **21**, 431–48.

DENNIS, R. W. G. (1938). A new test plant for potato virus Y. *Nature, Lond.*, **142**, 154.

GARNER, W. W. & ALLARD, H. A. (1920). Effect of length of day and other factors of the environment on growth and reproduction in plants. *J. agric. Res.* **18**, 553–606.

—— —— (1923). Further studies in photo-periodism. The response of the plant to relative length of day and night. *J. agric. Res.* **23**, 871–920.

SALAMAN, R. N. (1933). Protective inoculation against a plant virus. *Nature, Lond.*, **131**, 468.

TINCKER, M. A. H. (1925). The effect of length of day upon the growth and reproduction of some economic plants. *Ann. Bot., Lond.*, **39**, 721–54.

EXPLANATION OF PLATES V AND VI

PLATE V

Fig. 1. Interveinal mottle on President potato induced by the strain of X obtained from Alpaca noza.

Fig. 2. Top necrosis of U.S.D.A. 41956 following a graft from Parcco hancco.

Fig. 3. Necrotic rings on Arran Victory leaf caused by systemic infection with the Parcco hancco virus—? virus C.

Fig. 4. Tuber of Cacho amajaya sprouted before harvesting.

Fig. 5. *Datura stramonium* infected with the virus recovered from Papa runtusa.

PLATE VI

Fig. 1. Veinal mottle and ruffling of Katahdin leaf due to systemic infection with the Chohuahineca virus.

Fig. 2. Top of a King Edward potato infected with the Chohuahineca virus.

Fig. 3. Chohuahineca virus on *Datura stramonium*: local lesions.

Fig. 4. Chohuahineca virus on *Datura stramonium*: systemic infection.

Fig. 5. Faint local lesions of the strain of X recovered on *Datura stramonium* from Alcca fina.

Fig. 6. Leaf of Luqui mari showing characteristic small necroses.

THE INTRACELLULAR INCLUSIONS OF SOME
PLANT VIRUS DISEASES

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(With Plates VII and VIII)

CONTENTS

INTRODUCTION

ALTHOUGH intracellular inclusions have not been found in all virus diseases, they seem to be specific to these diseases and, in some, they are sufficiently frequent and characteristic to be of diagnostic value. Their production depends more on the infecting virus than on the species of host. For example, they have been found in a number of different species infected with tobacco mosaic virus, but not in any of the same species infected with potato virus Y or cucumber virus 1. It is not doubted that they are formed as a result of virus infection, but different workers have held widely divergent views as to their nature and significance. Some have regarded them as amoebae or as stages in the life history of a causative organism, to which names have occasionally been given, while others have regarded them simply as masses of coagulated cytoplasm.

With animal viruses it is more generally believed that the inclusion bodies are aggregates of virus particles. The use of ultra-violet light and microscopes of high resolving power has shown that the inclusions accompanying infection with some of the larger viruses contain numbers of elementary bodies with approximately the same size as that estimated for the virus particles by filtration experiments.. However, no adequate reason has yet been advanced to explain the aggregation of virus particles

which form stable suspensions *in vitro* into such characteristic bodies in the infected cells.

It has been found that plants infected with certain viruses contain proteins not present in healthy plants. These proteins have the characteristic properties associated with the different viruses, changes in them result in loss of infectivity, and it is highly probable that they are the viruses themselves. Beale (1937) has pointed out some features that one type of inclusion has in common with the protein isolated by Stanley (1936) from plants infected with tobacco mosaic virus. In this paper further similarities and differences between the behaviour of the inclusions and the purified virus preparations are described.

THE NORMAL CELL

Since a number of cell constituents have been described by various workers as possible causes of virus diseases or as virus inclusion bodies, a brief account of a normal adult solanaceous cell is given. The walls of parenchymatous cells are reinforced with cellulose and are birefringent. The thickness of the cellulose layer varies in different tissues, being thin in pith and cortical cells, and thick and reinforced with cutin in the outer walls of the epidermal cells and in the hairs. The cell contains cytoplasm and nucleus with cell sap occupying the central vacuole. The cytoplasm forms a continuous layer inside the wall, and strands cross the vacuole. It streams continuously around the cell, its movement being obvious because of the chondriosomes, plastids, oil globules and other particles embedded in it. The nucleus, an oval or spherical body containing one or more readily distinguished nucleoli, is also embedded in and carried round by the cytoplasm. Indications of the chromatin reticulum can sometimes be seen, but this is more obvious after fixing and staining. Most cells contain only one nucleus, but occasionally, e.g. in some adult pith cells, two are found. Sometimes, as in the formation of necrotic lesions in *Nicotiana glutinosa*, the binucleate condition is a result of virus infection.

In addition to the substances in solution, the sap and cytoplasm contain a number of crystals, globules and particles (Pl. VII, figs. 1, 2). The commonest crystals appear to be octahedra; these are inorganic, readily dissolve in hydrochloric acid, and are probably calcium oxalate. They vary in size and occur most frequently in the epidermis over the veins, where from one to a dozen or more can usually be found in a single cell. Globules, giving the staining reactions of both oils and proteins, occur in some plants, especially in the younger tissues (Sheffield, 1933),

and alkaloids occur in solution. Of most interest and frequent occurrence are particles, many of which superficially resemble small bacteria, varying in length from about 3μ down to limits of resolution. They can be found in large numbers in almost all cells and show active Brownian movement, but no autonomous movement. They vary in shape and perhaps in chemical composition, but they are too small for successful microchemical examination; some are definitely rod-shaped, while others are elliptical and a few are angular plates. When the cell dies they aggregate, and the addition of fixatives also often causes them to clump together. The larger of the particles are definitely birefringent with straight extinction, and when the cell is examined between crossed Nicol prisms they repeatedly appear and disappear as Brownian movement alters their position to the plane of polarization. It is possible that these particles are similar to the larger birefringent bodies described by Schmitt & Johnson (1938) in the microspores of *Tradescantia*, which they suggest are storage proteins. Bacteria often occur on the surface of hairs and epidermal cells, but we have never seen any in the cells of healthy or mottled plants. As these particles more nearly resemble bacteria than any of the other cell constituents it is possible that they are the short rod-shaped bacteria described by Iwanowski (1903) which formed apparent zooglea when fixed.

CELLS INFECTED WITH STRAINS OF TOBACCO MOSAIC VIRUS

Except in plants such as *Nicotiana glutinosa* which react to infection by the formation of necroses and have their cell contents destroyed, all the constituents described in healthy cells can also be found in infected cells, although the amount of cytoplasm often seems to be reduced considerably. The microscopic appearance of infected cells varies with the external symptoms, and depends on the strain of the virus and on the age of the tissues. If old leaves are infected, or a masked strain of the virus used, there are no external symptoms nor any definite microscopic changes in the cells. In mottled leaves some hypoplasia or hyperplasia occurs and abnormalities of the chloroplasts can be seen. Cytoplasmic streaming often seems to be increased early in infection, and greater numbers of the crystals and particles found in healthy plants may be seen.

The greatest difference between the healthy and infected cell is the formation of the characteristic inclusions in the latter. As early as 1903 Iwanowski described two main types in tobacco mosaic plants, one consisting of amorphous material, later named X-bodies by Goldstein (1924), and the other flat crystalline plates. The three strains of tobacco

mosaic virus that we have used mainly, those causing common tobacco mosaic, Enation mosaic and Aucuba mosaic, all cause the formation of the two kinds of inclusion. No significant differences have been found between the crystalline inclusions produced by the three strains (Pl. VII, figs. 3a, 4a; Pl. VIII, fig. 2), but the amorphous body of Aucuba mosaic differs in some respects from those of tobacco mosaic and Enation mosaic.

The amorphous[1] bodies of all three diseases are relatively stable entities, are preserved by ordinary cytological fixatives and give all the usual protein reactions. Those of tobacco mosaic and Enation mosaic, diseases with closely similar external symptoms, are small and superficially resemble amoebae. They average about 10μ in length, contain vacuoles, chondriosomes and oil globules, and as they are carried round the cell by the streaming cytoplasm frequently change their shape. The amorphous body of Aucuba mosaic, a disease readily distinguished from the other two, is larger, somewhat more granular and less like the surrounding cytoplasm. In some hosts the body is rather diffuse, but in others, especially *Solanum nodiflorum*, it may be spherical with a diameter of 30μ in large cells. There is no reason to assume that there is any essential difference between the amorphous bodies produced by the three virus strains, but we have been unable to follow the method of formation and disappearance of the amoeboid X-bodies as has been done in detail with the bodies of Aucuba mosaic (Sheffield, 1934).

At the time that external symptoms of Aucuba mosaic become obvious, minute particles containing protein appear in the circulating cytoplasm. These are carried round the cell and on coming together fuse. The aggregation continues until all the particles are joined together into one body. The outside layer will be subjected to rather different surface tension forces from the material inside the body, and this may be responsible for the properties of the body suggesting an external membrane (Goldstein, 1926; Sheffield, 1939). After some time the body degenerates and its place is taken by crystalline plates.

Over periods of some years the type and relative proportions of different kinds of inclusions produced by Aucuba mosaic virus have altered appreciably. Some years ago in *S. nodiflorum* amorphous bodies were produced in large numbers early in infection, and crystalline plates appeared to be formed only from disintegrating bodies. Crystal-like spikes, often as long as the cell, were also frequently seen (Henderson Smith, 1930; Sheffield, 1931). Recently we have found no spikes and fewer

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[1] The term "amorphous" in describing inclusions is used as opposed to "crystalline".

amorphous bodies, but large numbers of crystals. Even when plants were infected in 1936 with virus from leaves dried in 1927 no spikes were found, but these plants contained large numbers of amorphous bodies. After this source of the virus had been subcultured continually for two years it also produced large amounts of crystalline material without first forming the bodies.

The crystalline plates may occur in large numbers either alone or in the same cells as the amorphous bodies. They are best examined in fresh living cells, because fixation destroys them or causes the production of numerous striations, an effect responsible for the name "striate material" given to this type of inclusion. Like the amorphous bodies the plates give protein reactions, but they are extremely fragile and either disappear or alter in appearance if the cell is injured in any way. They are colourless and transparent, and have a refractive index higher than the cell sap. They are true crystals with a three-dimensional regularity, and as they slowly move around the cell and turn over they show both side and end faces. Faint striations can often be seen in the side faces of untreated crystals, especially if they are examined in polarized light. When seen in the basal plane some plates are regular hexagons, with all their angles 120° (Pl. VIII, fig. 2). More often they are irregular with only an occasional angle, suggesting that they are formed by unequal growth or by the fusion of separate crystals. Early in infection several crystals can often be found in one cell, but later they coalesce into a single, rather shapeless mass.

When seen edgeways they are oblong, and between crossed Nicol prisms are birefringent with straight extinction. They are not birefringent when examined flat (Pl. VII, figs. 3a–4b). The refractive index is greater along the thickness than along the length of the crystals, and as some are definitely hexagonal it is highly probable that they are hexagonal crystals. However, this could not be confirmed, since we were unable to obtain an interference figure, probably because of the small size of the crystals and because of the presence of the birefringent cell wall which greatly affects a critical examination in polarized light.

The characteristic inclusions cannot be found at all times in infected plants. In good growing conditions the first microscopic changes can be seen a week or so after infection when external symptoms become evident in the young leaves. The formation of the amorphous bodies of Aucuba mosaic commences at this time, and a few X-bodies and a little crystalline material can be found in plants with Enation or tobacco mosaic. The inclusions rapidly increase in number, reaching a maximum about a

month after infection. After a further month the amorphous bodies of Aucuba mosaic disintegrate and form crystals, and those of tobacco mosaic disappear. The crystalline inclusions persist for longer than the amorphous bodies, but after a few months both types have usually disappeared.

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COMPARISON OF THE INTRACELLULAR INCLUSIONS WITH PURIFIED
PREPARATIONS OF THE STRAINS OF TOBACCO MOSAIC VIRUS

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Purified preparations of tobacco mosaic type viruses have features in common with crystalline materials. They give an X-ray pattern and are birefringent (Wyckoff & Corey, 1936; Bawden et al. 1936), but no visible preparations have yet been made in vitro which are true crystals. The particles in the purified preparations are greatly elongated and, therefore, readily orientated. Solutions have a characteristic sheen; when dilute they show the phenomenon of anisotropy of flow strongly, and when concentrated they are liquid crystalline. Increasing the solid content by evaporation or by high-speed centrifugation causes the formation of anisotropic gels (Bawden & Pirie, 1937 a). Precipitating the virus with acid or ammonium sulphate greatly increases the sheen and produces birefringent needle-shaped bodies. These are fibrous in appearance, have pointed ends and no facets, but have been called crystals (Stanley, 1937). Bernal & Fankuchen (1937) have shown that the constituent particles in the needles are arranged parallel one to the other without any regularity of arrangement in the direction of their length. They are therefore not true crystals, but are a form of liquid crystal and are more accurately called paracrystals or microtactoids.

Iwanowski (1903) and Goldstein (1924) found that the addition of acid to the crystalline intracellular inclusions caused them to break down into needles. Beale (1937) has pointed out that in appearance these needles closely resemble the paracrystals of the purified virus. She also found that the pH stability range of the needles in the cells was approximately that of the virus in vitro, and it seems highly probable that the crystalline inclusions are rich in virus.

Purified tobacco mosaic virus is miscible with water in all proportions and does not settle out in a solid form when solutions are concentrated by evaporation. The fluids become increasingly viscous, and when the solid content is sufficiently great they turn to gels. A solid, or quasi-solid phase, the paracrystals, separates from solutions only if acid is added to reduce the pH to below 4 or if much salt (1/5th saturation with ammonium sulphate) is added. As the virus content of fully infective sap is only

about 0·3 %, the *p*H of cell sap is above 6, and the salt content is too small to precipitate the virus, it is highly improbable that the crystalline inclusions are deposits of pure virus, for there is no apparent reason for pure virus to settle out in these conditions. It is perhaps more probable that they are composed of an insoluble complex, formed by the union of the virus with some constituent or constituents of the host.

The purified virus readily unites with some protamines and histones, the one most studied being clupein (Bawden & Pirie, 1937 *a*, *b*), to form complexes whose behaviour in many ways resembles that of the crystalline inclusions. They are insoluble in dilute salt solutions near neutrality, conditions probably obtaining in the cells of young plants with a high water content. When a neutral solution of clupein sulphate is added to preparations of any of the strains of tobacco mosaic virus a precipitate with a pronounced sheen immediately separates. The precipitated material consists of fibres which microscopically closely resemble the paracrystals produced with acid or strong salt solutions, but some are longer and appear to be similar to the mesomorphic fibres which Best (1937) described as settling out from samples of clarified infective sap after keeping for some months (Pl. VIII, fig. 1). The insoluble complexes contain less than 5 % of clupein, apparently too little to affect the orientation of the particles. Their solubility varies greatly with small changes in *p*H and salt content, and also depends on the virus strain and on the relative amounts of virus and clupein in the system. The complexes all dissolve in salt solutions more concentrated than *N*/10, but the minimum amount of salt required for solution varies with the *p*H and is more at *p*H 5·5 than at *p*H 6. The stability of these precipitates is therefore similar to that of the crystalline inclusions which either dissolve or break down into needles if the cell is injured or has its *p*H altered by the addition of acid. If the crystalline inclusions are complexes of this type their formation in young tissues and their disappearance in plants infected for long periods can be explained on the basis of changes in *p*H and salt content which occur with increasing age. The sap of young plants has a relatively high *p*H value and low salt content. In these conditions such complexes would be insoluble and readily settle out, whereas with increasing acidity and salt content they would become increasingly soluble and tend to disappear.

There is one important difference between the artificial complexes produced with the purified viruses and the inclusions formed in the plants, for the former are paracrystalline whereas the inclusions are true crystals. This may merely mean that correct conditions for the production

of true crystals *in vitro* have not yet been found. However, at the present time it seems that the apparent difference in the behaviour of the virus in the plant and after purification is more probably a result of changes in the physical state of the virus brought about by the processes of purification. Precipitation of the virus in sap from young infected plants with acid or salts and resolution causes a reduction in the filterability and infectivity, and an increase in the anisotropy of flow. These results suggest that precipitation has increased the size of the infecting units by causing the virus particles to aggregate linearly (Bawden & Pirie, 1937 *a*). It seems that the virus particles as first formed in the plant are relatively small, and when rendered insoluble can arrange themselves regularly in three dimensions to form true crystals, but that the small particles readily come together to form greatly elongated micelles in which state they produce liquid crystals.

As precipitation of the virus *in vitro* has such a definite effect on its physical properties, if the crystalline inclusions are largely composed of virus it might be expected that precipitation in the plant would also have some effect. There is now a good deal of evidence that virus units of different sizes do occur in clarified infective sap. Wyckoff (1937) finds only one sedimentation constant (174×10^{-13} cm. sec.$^{-1}$ dynes^{-1}) for the virus from plants infected for short periods, but sap from plants infected for a month, or sap treated chemically, readily gives a second larger component with a sedimentation constant of 200×10^{-13}. A similar effect is also found in the optical properties of clarified infective sap. Sap from plants infected for short periods shows little or no anisotropy of flow when shaken between crossed polaroid plates unless the virus is first precipitated with acid or ammonium sulphate. With increasing length of infection period the amount of anisotropy of flow increases, and clarified sap from plants infected for a few months shows the phenomenon strongly, and little increase is obtained by precipitating and redissolving the virus. The larger virus particles therefore do not seem to occur until the crystalline inclusions are formed, and the proportion of these particles present in sap increases with the formation and resolution of the inclusions. The filtration results of Smith & MacClement (1938) also supply further evidence for particles of different sizes in infective sap, for they find that tobacco mosaic virus at different times has filtration end-points of 50 and 180 mμ. However, as the conditions determining the end-point are not given, these results cannot be correlated with the formation of crystalline inclusions.

The amorphous inclusion body is at first sight more difficult to connect

directly with the virus, but the formation of crystals, indistinguishable in appearance and behaviour from the crystalline inclusions, within degenerating bodies of Aucuba mosaic, suggests that the two types of inclusion have a common constituent. Also, Sheffield (1939) has shown that the isolated and carefully washed bodies from Aucuba mosaic are infective, and they therefore contain virus. As they also contain chondriosomes and oil globules, this cannot be taken as proof that the virus is an essential part of the body for it may have been adsorbed during the formation and circulation of the body around the cell. However, as the estimated weight of such inclusions is little greater than the minimum weight of purified virus necessary to cause infection, it seems that their infectivity is too great to be explained merely by the adsorption of virus. If these bodies are insoluble virus-host complexes, their amorphous nature, greater stability and different solubility indicate that the virus is combined with a constituent of the host different from that suggested for the crystalline inclusions, and that the ratio of this constituent to virus is greater than in the crystalline inclusions.

Insoluble virus-protein complexes, probably containing less than 50 % of virus, and superficially resembling the inclusion bodies, are produced when preparations of strains of tobacco mosaic virus are mixed with their antisera. Amorphous precipitates settle out which are much more stable than the paracrystalline precipitates produced by clupein, and which are unaffected by the addition of acid and are insoluble in salt solutions. If the virus-antiserum mixtures are kept circulating by convection currents the sequence obtained also closely resembles that of the formation of the body of Aucuba mosaic. As soon as the antiserum and virus have become mixed small particles appear throughout the fluid. In moving around these come into contact and fuse together until, finally, all the precipitate settles out in a fluffy mass. The precipitate is only formed if antibody and antiserum are present in correct proportions and its formation is greatly inhibited by the presence of too much virus. Also, although the solubility of virus-antibody complex is unaffected by small changes in pH or salt content, the precipitate dissolves fairly readily if excess virus is added. If the amorphous inclusions are virus complexes with solubility relationships similar to those formed by the union of virus and antibody, their formation early in infection, when the virus content of sap is low, and their disappearance later with increasing virus content could be explained.

It is not intended to suggest that the amorphous inclusions are produced by a union of virus and antibody in the plant, for there is no valid

evidence that plants contain or produce antibodies, and the analogy is put forward only to show that the apparent behaviour of the virus in the plant can be closely simulated *in vitro*. However, it is possible that the formation of inclusions does in part act as a protective mechanism, for it is difficult to imagine any virus that may be rendered insoluble in the inclusions possessing great biological activity.

Because of its greater stability the amorphous inclusion is more suitable for microchemical tests than the crystalline inclusion. It gives all the protein reactions of the purified virus, but there is no critical chemical test for detecting small amounts of the virus. Bawden & Pirie (1937 a) have shown strains of tobacco mosaic virus to be nucleoproteins, differing from the nucleoproteins characteristic of nuclei in that the nucleic acid contains ribose instead of a desoxy pentose. Feulgen's reagent readily identified desoxy pentose, but, unfortunately, there is no simple colour test for detecting nucleic acids of the ribose type. The amorphous body does not contain a desoxy pentose, and staining with Feulgen's reagent sharply distinguishes it from the nucleus, for the body is unaffected whereas the nucleus takes on a deep red or purple colour.

It is generally accepted that virus-infected plants contain intra-cellular inclusions only when they are showing external symptoms, and in plants infected with a masked strain of tobacco mosaic virus Beale (1937) found inclusions only in the occasional chlorotic areas. She suggests that the formation of inclusions is dependent on the concentration of the virus, and that only in the chlorotic areas is there sufficient virus for it to crystallize. Although this suggestion cannot be disproved there is some evidence against it. Merely increasing the virus content of purified preparations, even to twenty or more times that of infective sap, does not cause the virus to settle out in solid form. Also, the inclusions tend to disappear late in infection when the virus content of sap is greatest, and the virus content of plants fully infected with the masked strain is probably greater than that of plants with tobacco mosaic when they first commence to form inclusions. If the inclusions are complexes of the type we have suggested their formation will depend on the presence in infected plants of materials which can unite with the virus and render it insoluble. The close association of inclusions with external symptoms might then be determined, not by the greater virus content of obviously diseased plants but by the fact that only these plants contain such materials. In other words, the substances with which it is suggested that the virus combines may not be normal constituents of cells but disease products, the presence of which is indicated by external symptoms.

INFECTIONS WITH VIRUSES OTHER THAN TOBACCO MOSAIC VIRUS

In addition to the strains of tobacco mosaic virus we have also worked with tobacco ring-spot virus, potato viruses X and Y, tomato Bushy stunt virus, Hyoscyamus virus 3, and cucumber viruses 1, 3 and 4. We have not found intracellular inclusions in plants infected with potato virus Y or with any of the cucumber viruses, and if they occur it must be either in different conditions or with much less frequency than with the others. Their apparent failure to produce inclusions might of course be a result either of differences between them and the other viruses or of differences in their host plants. With potato virus Y and cucumber virus 1 it cannot be attributed to an effect of the host plants, for they infect a number of solanaceous species in which other viruses produce numerous inclusions. Nothing is known of the chemical nature of these two viruses or of the conditions causing them to precipitate, and, at the present time, their different behaviour in the plant cannot be correlated with any properties of the viruses *in vitro*.

It seems more probable with cucumber viruses 3 and 4 that the absence of inclusions may be an effect of the host plant. No chemical differences have been found between these viruses and tobacco mosaic virus, and only slight differences in their physical properties. They have antigens in common with tobacco mosaic virus, and *in vitro* precipitate in the same liquid crystalline forms and under closely similar conditions (Bawden & Pirie, 1937 *b*). Only members of the Cucurbitaceae have been infected with cucumber viruses 3 and 4, and as these are immune to tobacco mosaic virus there are no common hosts known in which the behaviour of the viruses can be compared. It is possible that cucumber plants do not contain materials capable of uniting with and precipitating the viruses, or alternatively, if complexes are formed, cucumber sap is so much more alkaline than tobacco sap that complexes insoluble in tobacco might be soluble in cucumber.

Results with tobacco ring-spot virus show that the host can determine the formation of inclusions and that cucumber is not a favourable host. In tobacco and other solanaceous plants this virus causes the production of large numbers of inclusions, the majority being amorphous and of the amoeboid form similar to those produced by tobacco mosaic virus. In addition, crystalline inclusions are produced; these are colourless and hyaline like those caused by tobacco mosaic virus, but they occur frequently (Pl. VIII, fig. 3). They also appear to have a different crystal form, for no hexagonal plates have been seen, the majority being

rectangular blocks which are birefringent when viewed along all axes. Infected cucumbers show severe symptoms, but of a different type from tobaccos. We have found no crystalline inclusions in infected cucumbers and only a few amorphous bodies. These are formed very infrequently and only in certain leaves, although the whole plants are severely diseased.

Potato virus X is a nucleoprotein forming liquid crystalline solutions and gels similar to those of tobacco mosaic virus, but when precipitated with acids, salts or clupein it is amorphous and not paracrystalline (Bawden & Pirie, 1938a). In keeping with this behaviour *in vitro* we have found only amorphous inclusion bodies in plants infected with this virus, although Clinch (1932) states that in one plant she found striate material. The inclusions are very similar to the X-bodies of tobacco mosaic, but differ from them in that they occur chiefly in the assimilatory tissues instead of in the hairs and epidermis.

Plants infected with Hyoscyamus virus 3 contain large numbers of amorphous inclusions in all tissues. These resemble the bodies of Aucuba mosaic both in appearance and in their manner of formation. We have found no crystalline inclusions, but when the bodies disintegrate they give rise to numerous long, very thin needles (Pl. VIII, fig. 4). These show no extinctions in a polarizing microscope, but whether this is because of their small size or because they are not birefringent is not known. Little is known about the chemical and physical properties of this virus; we have obtained preparations showing anisotropy of flow, but the precipitates from these with strong salt solutions were amorphous.

Tomato bushy stunt virus has also been shown to be a nucleoprotein, but to differ in many ways from the other viruses that have been isolated. Solutions are isotropic and, when precipitated with salts or with clupein sulphate, the purified preparations crystallize in the form of rhombic dodecahedra (Bawden & Pirie, 1938b). In many plants suffering from bushy stunt we have been unable to find any definite inclusions. In a few we have found amorphous bodies of the tobacco mosaic type (Pl. VIII, fig. 5) and all contain much crystalline material. Some of the crystals appear to be dodecahedra and have not been seen in healthy plants. However, as the crystals and birefringent particles of uninfected cells often occur in greatly increased numbers and the apparent dodecahedra are found only occasionally, it is impossible to be sure that these are true virus inclusions.

SUMMARY

The contents of healthy cells and those infected with a number of different plant viruses are described. Some of these viruses apparently do not cause the production of intracellular inclusions; others cause the production of amorphous bodies only, and the remainder produce both amorphous and crystalline inclusions. The properties of the inclusions are compared with those of purified preparations of the viruses. It is shown that insoluble complexes of the viruses with protamines, histones and proteins which in many ways resemble the intracellular inclusions can be produced *in vitro*. Possible explanations for the formation and disappearance of the inclusions in infected plants are suggested.

REFERENCES

BAWDEN, F. C. & PIRIE, N. W. (1937*a*). *Proc. roy. Soc.* B, **123**, 274.
—— —— (1937*b*). *Brit. J. exp. Path.* **18**, 275.
—— —— (1938*a*). *Brit. J. exp. Path.* **19**, 66.
—— —— (1938*b*). *Brit. J. exp. Path.* **19**, 251.
BAWDEN, F. C., PIRIE, N. W., BERNAL, J. D. & FANKUCHEN, I. (1936). *Nature, Lond.*, **138**, 1051.
BEALE, H. P. (1937). *Contr. Boyce Thompson Inst.* **8**, 413.
BERNAL, J. D. & FANKUCHEN, I. (1937). *Nature, Lond.*, **139**, 923.
BEST, R. J. (1937). *Nature, Lond.*, **139**, 628.
CLINCH, P. (1932). *Proc. R. Dublin Soc.* **20**, 143.
GOLDSTEIN, B. (1924). *Bull. Torrey Bot. Cl.* **51**, 261.
—— (1926). *Bull. Torrey Bot. Cl.* **53**, 499.
IWANOWSKI, D. (1903). *Z. PflKrankh.* **13**, 1.
SCHMITT, F. O. & JOHNSON, G. T. (1938). *Ann. Mo. bot. Gdn*, **25**, 455.
SHEFFIELD, F. M. L. (1931). *Ann. appl. Biol.* **18**, 471.
—— (1933). *Ann. appl. Biol.* **20**, 57.
—— (1934). *Ann. appl. Biol.* **21**, 430.
—— (1939). *Proc. roy. Soc.* B. In the press.
SMITH, J. H. (1930). *Ann. appl. Biol.* **17**, 213.
SMITH, K. M. & MacCLEMENT, W. D. (1938). *Proc. roy. Soc.* B, **125**, 291.
STANLEY, W. M. (1936). *Phytopathology*, **26**, 305.
—— (1937). *Amer. J. Bot.* **24**, 59.
WYCKOFF, R. W. G. (1937). *J. biol. Chem.* **121**, 219.
WYCKOFF, R. W. G. & COREY, R. B. (1936). *J. biol. Chem.* **116**, 51.

Fig. 1.

Fig. 2.

Fig. 4 a.

Fig. 3 a.   Fig. 3 b.   Fig. 4 b.

BAWDEN and SHEFFIELD.—The Intracellular Inclusions of some Plant Virus Diseases (pp. 102–115)
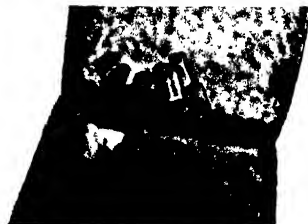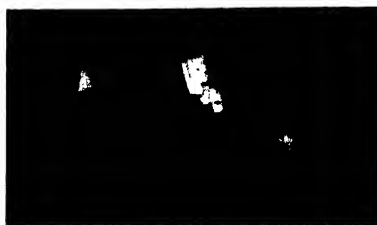
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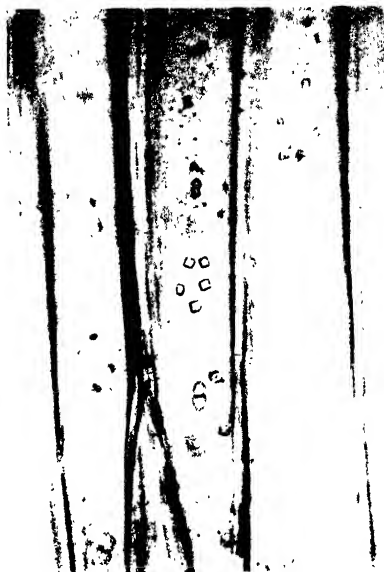
Fig. 3.

Fig. 2.

Fig. 4.

Fig. 5.

EXPLANATION OF PLATES VII AND VIII

Some of the conditions discussed have already been recorded photographically, and are figured in the papers of Beale, Clinch, Iwanowski and Sheffield cited.

The photographs were taken with a Leitz "Makam" camera. For Plate VII, figs. 1 and 2, a Leitz apochromatic 2 mm. objective (N.A. 1·4) was used in conjunction with a Leitz 10 × periplanatic ocular, giving a magnification of 900. For the other figures, a Leitz 6L achromatic objective (N.A. 0·65) was used together with a 10 × periplanatic ocular giving a magnification of 450. Contact prints are reproduced without alteration in size.

All preparations were unstained, and except for Plate VII, figs. 1 and 2, the cells were living when photographed.

PLATE VII

Figs. 1, 2. Healthy *Nicotiana tabacum*. Epidermal cells from beneath a vein. The vacuoles of both cells contain numerous particles many of which are rod-shaped and bear a superficial resemblance to bacteria. In addition fig. 2 also shows octahedral crystals, probably calcium oxalate. (These preparations were first examined in the living condition, but Brownian movement was too great for accurate photographs. Acetic alcohol was therefore run under the cover glass and the photographs taken immediately the cells were fixed. The process of fixation was closely watched but except for a slight shrinkage of the cytoplasm no artefacts were observed.)

Figs. 3 *a*, *b*. Hair of *Lycopersicum esculentum* infected with Enation mosaic virus. In the upper cell an irregular crystalline inclusion is seen lying flat; in the lower cell a similar inclusion is seen edgeways. Fig. 3 *a* taken with transmitted light and fig. 3 *b* in polarized light. The cross cell wall and the inclusion lying edgeways are birefringent, but the inclusion lying flat is not.

Figs. 4 *a*, *b*. Hair cells of *Solanum nodiflorum* containing crystalline material produced from disintegrating amorphous inclusions. Fig. 4 *b* photographed between crossed Nicol prisms. The crystals in the upper cell are lying edgeways and are birefringent.

PLATE VIII

Fig. 1. Insoluble mesomorphic fibres, produced by the addition of a neutral solution of clupein sulphate to a purified preparation of Aucuba mosaic virus.

Fig. 2. Hair cell of *Nicotiana tabacum* infected with tobacco mosaic virus. Two plate-like crystals are seen, one being almost a perfect hexagon and the other slightly irregular.

Fig. 3. *N. tabacum* infected with tobacco ring-spot virus. Epidermal cells from beneath the vein containing numbers of small crystalline blocks.

Fig. 4. Hair cell of *N. tabacum* infected with Hyoscyamus virus 3. The amorphous inclusion is disintegrating and producing long needle-like fibres.

Fig. 5. *Lycopersicum esculentum* infected with the bushy stunt virus. Two hair cells are seen each containing a single amorphous inclusion body.

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STUDIES ON APHIDES INFESTING THE POTATO CROP

VII. REPORT ON A SURVEY OF THE APHIS POPULATION OF POTATOES IN SELECTED DISTRICTS OF SCOTLAND (25 JULY–6 AUGUST 1936)

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(With 1 text-figure)

CONTENTS

INTRODUCTION

THE results of the survey of the aphis population on potato crops in selected districts in Scotland cannot be fully understood without a brief summary of the findings of the ecological studies on the aphis problem which led to these extended investigations.

Problem in north Wales. In north Wales there are two different types of district, one in the eastern portion where the spread of virus infection has been proved to be rapid—amounting to 33% infection developing within two years in initially healthy stocks (Currie, 1933); the other, mainly in western districts, where, with roguing, there has been no increase in virus infection among the potato stocks since the establishment of the Welsh Seed Potato Scheme in 1928. It has been the purpose of the entomological studies to compare the aphis population in these two areas, in which it has been found there is a striking contrast (Davies, 1934). The standard method, which was devised for these comparative studies, is to count the number of aphides per 100 (lower) leaves, taken at random as the crop is traversed to and fro, as the index figure of the aphis population. By this method it was found that the index figure for the important insect vector *Myzus persicae* Sulz. always exceeded 100 and more commonly approached 1000 vectors/100 leaves in the district where the spread of viruses was rapid. On the other hand, in areas where little or no spread occurred, the number of *M. persicae*/100 leaves seldom exceeded 20, and often none was recorded. It was found that the optimum period for these surveys was mid-July, when the aphis population was usually approaching a maximum. Surveys in June and September were practically useless.

Experimental results. Having established this difference in the population in the two districts, it was necessary to ascertain what factors were responsible for the striking contrast occurring in districts not more than 100 miles apart.

The effect of temperature upon insect activity is well known and, since it was found that winged *M. persicae* fly readily at temperatures above 65° F., it was evident that such temperatures would be prevalent, in both districts, on most days in June and July when the aphides were migrating.

The influence of humidity upon aphis migration was unknown, but the mean monthly figure for relative humidity in the two districts contrasted markedly: the more humid districts of south Caernarvonshire, with lichen and moss-covered trees and sea mists, having a much higher figure than the drier districts of Flintshire (Davies, 1935 a). Experiments

were, therefore, carried out to ascertain the effect of humidity upon the flight of these insects. In the drier atmospheres the aphides flew almost incessantly, but flight ceased in the higher humidities above 80 % (Davies ,1935*a*).

Experiments were next undertaken to find out the effect of wind velocity upon migration (Davies, 1936), and it was surprising to discover that, whereas the aphides flew continuously when the air was still, the flight of *M. persicae* ceased completely as soon as the wind velocity reached only 4 m.p.h.

By means of a mechanical insect trap (Davies, 1935*b*), revolving and catching insects continuously near the meteorological station at the College Farm near Bangor, it has been possible to study the effect of combined meteorological factors upon migration. The results up to the present time indicate that aphis migration only attains appreciable intensity under the following combination of meteorological factors, viz. when the temperature exceeds 70° F. with a wind (or rather light breeze) velocity below 5 m.p.h., and when, in north Wales, these come from an easterly direction, from which point of the compass all breezes are dry.

Differences in meteorological conditions in the two areas, therefore, partly explained the contrast in aphis population on the respective potato crops, and there can be no doubt that the frequency of favourable weather conditions for flight during late May and June, when the aphides are flying to the potato crops, and again in September, when they are flying back to the winter hosts, has a very marked influence on the subsequent population of aphides on potatoes.

Importance of winter host plants. Meteorological differences did not prove to be the whole story, however, and a careful study of the life history of the aphis vector was necessary. It will be clear that with an annual crop such as potatoes, which is free from aphides when it appears above ground (Davies, 1932), the source of the aphides *before* they arrive on the potatoes will have an important bearing on the aphid population. *M. persicae*, like many other aphides, is known to over-winter in the egg stage on hardwood trees, in this case nectarines and peaches.

It was obvious from the distribution of the aphis in early summer that these plants could not account for all winged forms which arrive on potatoes in June. A search was made for other possible hardwood hosts but without success. Then a chance inspection of savoy cabbages at a provincial market in December showed them to be literally smothered with wingless *M. persicae*. Subsequent examination has proved that cruciferous plants, particularly winter Brassicae, harbour large numbers

of this species of aphis. In north Wales, at least, there can be no doubt that such plants as savoys, cabbages, brussels sprouts, swedes and turnips afford the main source of this insect vector in spring (Davies, 1934). Although the actual individuals arriving on Brassicae from potatoes in October die before spring there is a gradual reproduction of wingless generations on these crops throughout the winter (Davies & Whitehead, 1935). On the advent of warm weather, reproduction increases rapidly, and quite early a few winged forms may be produced which fly to seedling charlock and other weeds. Then the main migration of winged forms from the cruciferous plants takes place in late May and June. These migrating alatae readily find even single potato plants and, subsequently, produce several generations of wingless forms (Davies & Whitehead, 1938). Even the wingless aphides have been found to be in constant movement within the potato crop (Davies, 1932), and this constitutes a factor of considerable importance in the spread of virus diseases within any one crop. When the potatoes mature in August and September, winged forms are produced on the dying potato foliage and then complete the life cycle by a flight to the winter host plants.

The importance, therefore, of proximity of winter Brassicae in determining the initial aphis population on potatoes becomes obvious. There is, indeed, ample evidence to demonstrate that in market-garden districts, or in the vicinity of towns and villages where there are many gardens, there is invariably a considerable increase in the aphis population of potatoes as compared with districts remote from Brassicae crops. The eastern districts of north Wales include market-garden areas and border on the large market-garden districts of Cheshire and Lancashire. Further, the drier easterly breezes come from these latter areas and thus, no doubt, contribute to the high aphis population of potatoes in the eastern districts of north Wales as compared with that found in the western areas.

THE PRESENT SURVEY

Aphis populations at each centre. The purpose of the survey in Scotland was to ascertain whether the results of the investigations in north Wales could be applied to other districts. Scotland was selected because of the importance of the potato crop in that country, and in order to note any differences which might be attributable to the more northerly latitude. In the time available (25 July–4 August) it was not, of course, possible to do more than study the aphis population in a relatively few selected areas. It was, therefore, decided to restrict the observations to the eastern areas of Scotland where the comparison with north Wales might

yield the more interesting data in view of the easterly sea breeze being laden with moisture in contrast to the dry easterly winds of north Wales.

The 1936 season was a rather late one, and it was this fact which decided the slightly delayed period (25 July–4 August) selected for the survey. Wherever possible an early and a late variety of potato were examined at each centre and an effort was made to include the same
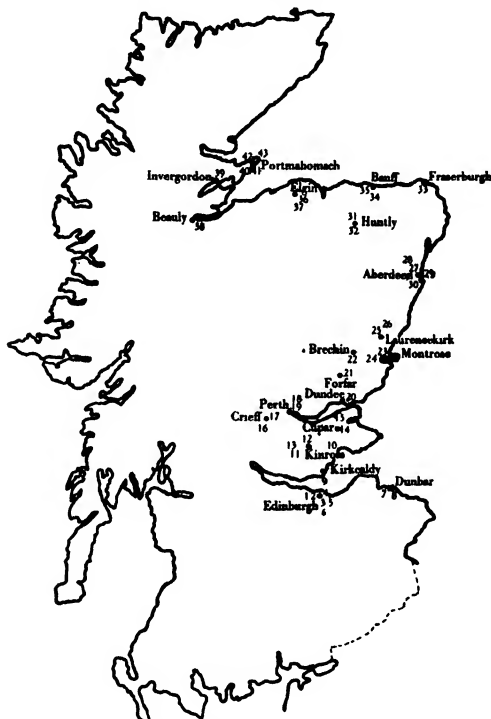
Fig. 1. Positions of centres in Scotland referred to in Table I.

varieties wherever a choice was available. This latter seemed advisable although it has been established (Whitehead *et al.* 1932) that there is no definite varietal selection by aphides, and that the time of appearance above ground, and the condition of the plants when migration takes place, are of greater importance than variety in determining the degree of aphis infestation.

The detailed results of the survey, with aphis species and population on each leaf examined at each centre, have been filed, and the accompanying table contains a summary of these observations. The positions of the centres in Scotland are shown in Fig. 1.

Table I. Data collected on survey

Centre	Location	Aspect	Variety	Number aphides/100 leaves	% leaves infested	Number M. persicae/100 leaves	Other spp./100 leaves	Remarks
			Midlothian 25 July 1936					
1	2 miles W. of Edinburgh	Open to W. & S. Easterly slope 200 ft.	Great Scot	5	3	5	—	—
2	Do.	200 ft. Open to E. Flat	Alness	19	6	19	—	—
3	Nursery E. of Edinburgh	264 ft. Many roses* growing during winter	Duke of York	361	90	100	258 *Mac. gei*	* Winter host of *M. gei*
4	2 miles E. of Edinburgh separated by slight hill	200 ft. In village. Flat	Great Scot	121	31	79	24 *Mac. gei* and 18 *M. pseudosolani*	—
5	2 miles E. of Edinburgh	100 ft. Hill 823 ft. to W. near housing scheme	Epicure	125*	70	90	35 *Mac. gei*	* On basis of 50 leaves
6	5 miles S. of Edinburgh	850 ft. Upland plateau with high Pentland hills to N.W., hills 600–800 ft. to N., other hills E. and S.	Eclipse / Arran Crest	8 / 1	2 / 1	2 / 1	6 *Mac. gei* / 0 *Mac. gei*	— / —
			East Lothian 28 July 1936					
7a	3½ miles S.W. of Dunbar	20 ft. Sea and marsh 1 mile to N.E., land rising to mountain 3 miles W.	Dunbar Cavalier	21	10	21	—	—
7b	Do.	Field more inland; swedes grown on adjacent field until late April –May when they had much foliage	Dunbar Yeoman	196*	50	186	—	* On basis of 60 leaves
8	5 miles S.E. of Dunbar	450 ft. Hills (900 ft.) N.W., short valley running E. to W. with mountains 1 mile to S.	Arran Banner	29	6	6	25 *A. rhamni*	—
			Fifeshire 26 and 27 July, 1936					
9a	2 miles S. of Kirkcaldy	200 ft. Field mainly open with hillock to side of crop. Sea 1 mile to E. and 2 miles S. Open land to W.	Doon Star / King Edward*	22 / 46	10 / 10	19 / 16	3 *A. rhamni* / 30 *A. rhamni*	* On basis of 50 leaves
9b	Do.	Field near shore with easterly slope. Sea ¼ mile E. and S. Well exposed	British Queen	5	5	5	—	—
10	1 mile S. of Ladybank and village of Kettle ¼ mile S.	150 ft. Undulating country to W. Sea 20 miles to E.	Sharpe's Express / Great Scot	67 / 56	28 / 32	28 / 25	31 *M. pseudosolani* / 19 *M. pseudosolani*	— / —

Table I (*cont.*)

Centre	Location	Aspect	Variety	Number aphides/100 leaves	% leaves infested	Number M. persicae/100 leaves	Other spp./100 leaves	Remarks
			Fifeshire (cont.)					
11	2¼ miles W. of Kinross	420 ft. Open plain	Catriona	1	1	0	0	—
			Arran Consul	1	1	1	0	—
12	Adjoining Auchtermuchty	300 ft. Many gardens to N.W.	Kerr's Pink	176	48	45	76 *Mac. gei*	—
13	2 miles W. of Milnathort	400–500 ft. Open district to E. and W.	Great Scot	1	1	0	1 *Mac. gei*	—
			May Queen	8	3	2	6 *Mac. gei*	—
14	4 miles N. of Cupar	100–200 ft. Open field in slight valley running E. to W. No villages near	Arran Pilot	10	2	2	1 *M. pseudo-solani*	6 *Mac. gei*
			Majestic	6	2	2	4 *M. pseudo-solani*	0 *Mac. gei*
15a	4 miles N.E. of Newport	500–600 ft. On steep slope of N.E. facing Firth of Tay	Duke of York	5	2	2	6	0
			Arran Pilot (500 ft.)	0	0	0	0	—
15b	Do.	Lowland fringe of Firth of Tay	Arran Pilot (600 ft.)	0	0	0	0	—
			Arran Pilot Ballydoon	6 4	2 2	6 4	—	—
			Perthshire 30 July 1936					
16	2 miles S. of Crieff	150 ft. Inland basin with high trees form ¼ mile circle around field. Mountains to W. and N.	Majestic	1	1	1	0	—
17	Adjoining Crieff	300 ft. Nursery S.E. of town with field sloping to S.E. Rose trees grown in quantity	Kerr's Pink	20	14	5	14 *Mac. gei*	—
18	2¼ miles E. of Perth	200 ft. On ridge running between Perth and New Scone	Great Scot	29	11	18	0	—
19	Adjoining Perth	100 ft. Alongside mansion and other gardens. Field sloping S.W.	Duke of York	215	36	113	95 *Mac. gei*	—
			Angus 31 July 1936					
20a	1 mile E. of Dundee	154 ft. Open field with housing scheme encroaching	Majestic	191	51	77	96 *Mac. gei*	—
20b	Do.	60 ft. S.E. fringe of Dundee among houses. Sea less than ¼ mile to E.	Ballydoon	394	64	302	92 *Mac. gei*	—
21	Dunnichen	380 ft. Flat field with open land to W. Hills rising to 500 ft. to S., nearest village 1 mile to E.	Sharpe's Express	10	5	51	9 *Mac. gei*	—

Centre	Location	Aspect	Variety (cont.)	Number aphides/100 leaves	% leaves infested	Number *M. persicae*/100 leaves	Other spp./100 leaves	Remarks
			Angus (cont.)					
22	5 miles N.W. of Brechin	436 ft. Upland plain running slightly down from S.W. to N.E. Hills and mountains to N. and plain to S. No villages near	Arran Chief Great Scot Kerr's Pink*	0 0 1	0 0 1	0 0 1	0 0 0	— — * Kerr's Pink grown at 352 ft. in field bordered by trees
23	Farnell	82 ft. Inland plateau well wooded in parts but open to Montrose Basin to E. and with large deer park to N. and broken forests to W. No villages near	Arran Banner Arran Pilot	0 4	0 2	0 4	0 0	— —
24	2 miles N. of Montrose	178 ft. Sheltered from town by slight hill. No villages near. Open plain with sea 3 miles to E. and S.E. Slight hills to N. Open 10–20 miles W. and S.W.	Ally Majestic	0 2	0 2	0 1	0 0	— —
			Kincardineshire 31 July 1936					
25	1½ miles N. of Laurencekirk	229 ft. Open plain sweeping at foot of Grampians	Majestic	3	2	3	0	—
26	Fordoun	250 ft. At foot of Grampians which run W. to N. Hills rising to 860 ft. to S. and E. Open plain to S.W. No villages near	Majestic Duke of York	2 9	2 1	1 0	0 9 *A. rhamni*	— —
			South Aberdeenshire 2 August 1936					
27	5 miles W. of Aberdeen	300 ft. In experimental plots. Hills rising to 800 ft. to N.W., W. and S.W. Open to N.	Arran Pilot Great Scot	15 21	5 7	15 18	0 0	— —
28	3 miles N.W. of Aberdeen	200 ft. Hills 280 ft. to S.E., undulating hills rising to 250 ft. to E. with sea 3 miles beyond	Arran Banner Majestic	2 3	3 3	2 2	0 0	— —
29	Eastern fringe of Aberdeen	20 ft. Sea ¼ mile to E.	Majestic	171	38	77	64 *Mac. gei* 38 *A. rhamni*	—
30	¼ mile S.W. of Aberdeen	100 ft. Slight hillock protecting from S.W. winds	British Queen	51	21	26	24 *Mac. gei*	—
			North Aberdeenshire 3 August 1936					
31	1 mile N.W. of Huntly	400 ft. Field in inland valley with forest and mountain to N. Hills rising to 700 ft. to W. and E. Field with S.E. slopes. Swedes grown in previous year	Golden Wonder Kerr's Pink	5 3	5 3	5 3	0 0	— —

Centre	Location	Aspect	Variety	Number aphides/100 leaves	% leaves infested	Number M. persicae/100 leaves	Other spp./100 leaves	Remarks
			North Aberdeenshire (*cont.*)					
32	1¼ mile S. of Huntly	420 ft. Market garden on S.W. slope at mouth of valley extending W., bounded by hills to N. and W. and slight hills to E.	Duke of York	42	13	36	0	—
33	4¼ miles S.W. of Fraserburgh	166 ft. Field sloping E. Sea 5 miles to N.E. and E. Hills sloping to 300 ft. to S. and W.	Duke of York Great Scot	0 0	* *	0 0	0 0	* 1 *M. persicae* taken on 20 leaves in gardens adjoining
			Banffshire 2 and 3 August 1936					
34	4 miles S.W. of Banff	350 ft. Open fields with sea 3 miles to N.E. Hills rising to 500 ft. ½ mile to S.W. and S.	Duke of York Majestic Kerr's Pink	0* 0* 0*	0 0 0	0 0 0	0 0 0	* 1 *M. persicae* taken on small patch of kale in adjoining garden
35	Boyndie	60 ft. Small valley facing N.E. with hills rising to 180 ft. to W. and 250 ft. to N.W. Sea 1¼ miles to N. Not typical of district	Duke of York	24	4	3	21 *A. rhamni*	—
			Morayshire 3 August 1936					
36	S.W. fringe of Elgin	37 ft. Adjoining numerous gardens. Kale in next field in 1935. Slight N.W. slope	Golden Wonder	901	76	829	68 *Mac. gei*	—
37	1¼ miles S.W. of Elgin	50 ft. Sheltered by woods on all sides. No cruciferous crops grown near during 1935	King Edward	58	13	27	31 *Mac. gei*	—
			Ross-shire 4 August 1936					
38	1¼ miles S.E. of Beauly	21 ft. On fringe of Beauly Firth. Hills rising to S.W., W. and N.	Golden Wonder Duke of York	6 6	5 6	4 4	0 0	— —
39	Invergordon	20 ft. On flat basin of Cromarty Firth ¼ mile to S. and E. Hills rising to mountains 2 miles to N. Hills to W.	King Edward Majestic	2 7	2 4	2 7	0 0	— —
40	2 miles E. of Portmahomack	109 ft. On peninsula exposed to sea to E. and W., land tapering to sea to N.	King Edward	6	4	5	0	—
41	1 mile S.E. of Portmahomack	100 ft. Open and exposed field	Great Scot	8	7	6	0	—
42	2 miles N. of Portmahomack	78 ft. On peninsula with sea to N. and E. and W.	Arran Pilot	5	2	4	4	—
43	Portmahomack peninsula	100 ft. 1 mile from apex of peninsula. Exposed	May Queen	3	2	3	0	—

DISTRIBUTION OF THE DIFFERENT SPECIES OF APHIDES

(a) *Myzus persicae* Sulz. Specimens of this important insect vector of virus diseases were found at all the forty-three centres visited. In two cases (centres 33 and 34) none was actually included in the sample of 100 leaves (which, however, is not intended to give an absolute figure of population), but individuals were found in adjoining gardens. This species was taken almost at sea-level and also at the highest centre visited (850 ft.); it was common both in the north and the south. The special problem of the association of *M. persicae* with winter Brassicae is dealt with later. The index figure of population ranged from 0 individuals/ 200 leaves at centres 15a, 22 and 34, to over 100 individuals/100 leaves at centres 3, 7b and 19; with exceptionally high figures of 302 at centre 20b, and 829 at centre 36.

The development of the life history of this species in the east of Scotland seems to be similar to that found in north Wales. Only fifteen of the 2154 *M. persicae* collected were winged forms and, since wing buds were present on other specimens, it suggested that even these fifteen alatae had been produced on the potato crop on which they were collected. These facts point to a main migration of winged forms having taken place in June or earlier and, as is the case in north Wales, these winged parents subsequently died by the end of July. The heavier infestation, in general, of the earlier maturing varieties this year also indicated an early migration of the aphis.

(b) *Macrosiphum gei* Koch. Although this species has been recorded as a vector of leaf-roll in potatoes in America (Schultz & Folsom, 1925) and Holland (Elze, 1927), it has failed to transmit the virus in experiments under glass in this country (Smith, 1929, 1931). *M. gei*, which is often very common on potatoes in this country, over-winters in the egg stage on roses, and is later in arriving on potatoes than is *Myzus persicae*.

It was recorded at twenty-four of the centres; in low and high altitudes, and in the north as well as in the south. The index figures ranged from 1 to 258/100 leaves; the latter figure being obtained at centre 3 where rose trees were grown in large numbers in the nursery.

(c) *Aphis rhamni* Boyer. This species, like the last one, is recorded as a vector of leaf-roll and mosaic of potatoes in U.S.A. and Holland, but there is no evidence of transmission by *A. rhamni* in this country (Smith, 1931). The frequency and the numbers of this species found on potatoes during the last few years indicate a need for further transmission studies in this country. Little is known of its life cycle in this country except

that the buckthorn, which is its normal winter host, is rare in north Wales, and could not supply the numbers of the species appearing on potatoes. However, its habit of remaining in colonies on the same leaf, rather than moving to other foliage, would probably reduce its importance as a vector of potato viruses, as compared with *Myzus persicae*. During the present survey it was taken at ten centres, but nowhere did it exceed 30 individuals/100 leaves.

(*d*) *Myzus pseudosolani* Theob. This species is stated (Smith, 1929, 1931) to be a vector of potato leaf-roll, and possibly also of potato mosaic. It is seldom present in large numbers and little is known of its life history. It was recorded during the survey at twelve centres, and only in one instance did it attain 31/100 leaves.

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SUBSEQUENT DEVELOPMENT OF APHIDES AT EAST CRAIGS
AND AINVILLE

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It was, of course, impossible to keep continuous observation on the aphis population at the Scottish centres, so that the writer is the more indebted to Mr George Cockerham for undertaking this task as regards East Craigs and Ainville near Edinburgh. It is clear from his observations that the few aphides present at the time of the visit of the writer in July continued to reproduce until September. The counts made on plots at East Craigs showed that maximum infestation was reached about 10 September when the index figure was 800 *Myzus persicae*/100 leaves. At Ainville the maximum infestation of 500/100 leaves was reached on 5 September. This late development of wingless forms *within* the crop would, of course, only be of importance in spreading any virus infection already existing in the crop. It would be of great interest to ascertain whether this late development is an annual feature in certain districts in Scotland; though in 1936, at least, it is clear that at centres 20*b* and 36 the aphis development had begun early. In north Wales a late development is characteristic of the seed-producing districts.

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RELATIONSHIP OF PROXIMITY OF WINTER HOSTS TO APHIS POPULATION

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The fact that *Myzus persicae* generally hibernates on winter Brassicae suggests the potential danger of growing potatoes, especially for seed, in the neighbourhood of such crops. Hence the importance of market-garden areas, villages, allotments, etc., which yield enormous numbers of winged forms in spring. This influence of proximity to gardens, etc., on the aphis population of potatoes was clearly demonstrated during the survey. Commencing in Midlothian, it will be seen from the tabulated

data that the heavy infestation at centre 3 occurred under nursery conditions where a quantity of winter hosts would be grown in close proximity. It will also be noted that this centre differed from all others in that *Macrosiphum gei* formed the predominant species; the winter host plant (roses) being grown in large numbers. The index figure for *Myzus persicae* (100/100 leaves) was also high owing to the proximity of winter Brassicae. In Fifeshire, the highest aphis populations were recorded at centres 10 and 12, which were in close proximity to villages. In Perthshire, the importance of this factor was again clear, for at centre 19, which bordered the east of Perth, 215 aphides were taken per 100 leaves as compared with 29 taken in a crop on a ridge 2 miles farther east. Even at Crieff, with its low infestation, a definite increase was found under market-garden conditions (compare centres 16 and 17).

In Angus the role of this factor was again evident, for at centres 20*a* and 20*b*, which are on the eastern outskirts of Dundee, index figures of 191 and 394 aphides/100 leaves were recorded, the higher figure being obtained practically in the town. In Aberdeenshire the story is continued, centres around Aberdeen being visited in order to test this factor. To the west of the city (centres 27 and 28) the aphis infestation was not very high (the index figures ranging from 3 to 21/100 leaves), but on the eastern border of the city (centre 29) 171 aphides were recorded per 100 leaves. The significance of the higher figure on the east at this and other centres will be referred to later. Even in an inland district like Huntly, the increase in aphides under market-garden conditions is seen in centre 32. In Morayshire, a further extreme case of the important influence of towns is seen at centre 36, which gave the highest figure found during the survey, for 901 aphides were taken on 100 leaves. The field at this centre extended almost to the town.

There can, therefore, be no doubt of the importance of market gardens and private gardens in determining the aphis population on neighbouring field crops of potatoes. This had already been found to be the case also in north Wales. As a basis of comparison it may be mentioned that in the market-garden areas near Holywell and Chester the total aphis population/ 100 leaves on 15 July 1936 amounted to 271 and 757 respectively. Again, confirmation was found at Ormskirk Seed Testing Station, in the centre of the market-garden area. Here, the phenomenal number of 1782 aphides/100 leaves (50 actually counted) was found on mixed varieties on 6 August 1936. Of these, 1092 were *M. persicae* and 690 *Macrosiphum gei*. Every lower leaf examined was infested and as many as 65 aphides were counted on a single leaf.

Another factor in connexion with proximity of winter host plants appeared to have greater importance in eastern Scotland than is the case in north Wales. Swedes and turnips in north Wales are not a potential danger as winter hosts because most of these crops are carted from the field and placed in pits, or fed off, before January. During the survey in Scotland, however, the higher infestation at centre 7 b, with no village near, required further investigation, and was only explicable in the light of the practice in this district—as in others—of leaving swedes, turnips and kale in the field until spring. Sometimes the practice of "laying", or "Scheuching" as it is called locally, is carried out, i.e. the swedes are thrown into rows and lightly covered with soil. Whether this is done or the roots are left in the field there is considerable growth of foliage in early spring, even extending into May, on which *Myzus persicae* could hibernate. Observations made during the survey suggested that the presence of the aphis vector on potatoes could often be correlated with that of cruciferous crops during the winter months. In view of the practice of growing "special stock" seed alongside or in the same fields as swedes, it would be of value to ascertain what part these crops really do play, in Scotland, in providing the initial population of *M. persicae* on potatoes in spring.

RELATIONSHIP OF METEOROLOGICAL CONDITIONS TO APHIS POPULATION

The effect of meteorological conditions upon the migration of aphides in a given district requires prolonged investigation but, from the studies carried out in north Wales which were reviewed in the Introduction, it seemed likely that the frequency of *dry, light* breezes with temperatures above 65° F. would determine the extent of the migration to potatoes in late May and in June. The meteorological records in Scotland for June 1936, when the main migration of winged aphides would take place, were examined to see if any correlation between meteorological factors and aphis population was apparent. For this purpose, centres where there was an ample supply of winter hosts, such as can be found in market gardens and villages, were selected. From field observations referred to previously, it was clear that only days on which the maximum temperature exceeded 65° F. should be considered and, of these, days with wind velocities below 5 m.p.h. and relative humidities below 75 % would be the occasions on which migration of winged aphides would occur. Unfortunately, nowadays, the available meteorological data generally include only wind and humidity records taken at 9 hr. G.M.T., which is not the

time of day when general aphis migration takes place. There are, however, certain indications in the studies made during the survey which seem worthy of record.

Centres of high aphis infestation

(i) *Centre* 36, with 901 aphides/100 leaves. The figures from the meteorological station at Forres show that, of the 43 visited, this centre had the highest number of days (18) in June with maximum temperatures above 65° F. and on 13 of these days the wind velocity at 9 a.m. was below 5 m.p.h. Further, it was found that, even so early in the day, the relative humidity was below 75 % on 13 occasions; this also being the highest number of such days found to have occurred at any centre visited.

(ii) *Centre* 20 *b*, with 394 aphides/100 leaves. Data from Dundee showed that this centre had 17 days in June with maximum temperatures above 65° F., and on 11 of these the wind velocity at 9 a.m. was below 5 m.p.h., whilst 12 of the 17 days also showed a relative humidity below 75 % at this hour.

(iii) *Centre* 3, with 361 aphides/100 leaves. This nursery on the east of Edinburgh was exceptional in that *Macrosiphum gei* predominated and, owing to the presence of rose stocks (its winter host) in the same nursery, it suggested a very local migration. Probably centres 4 and 5 (with 121 and 125 aphides/100 leaves respectively) are more representative of fields within urban areas. The meteorological data from Edinburgh, approximately 2 miles to the west of these three centres, showed that on 10 days in June the maximum temperature exceeded 65° F., and of these 9 had wind velocities below 5 m.p.h. at 9 a.m., and 6 days had a relative humidity of less than 75 %.

(iv) *Centre* 19, with 215 aphides/100 leaves. Data from Perth showed that on 16 days in June the maximum temperatures exceeded 65° F., and on 10 of these the relative humidity was below 75 % at 9 a.m. There were, however, only 5 days when the wind velocity was below 5 m.p.h., and therefore suitable for aphis flight. On the other hand, this centre adjoined a number of gardens, and was itself sheltered, so that conditions were optimum for the rapid breeding up of the progeny of such aphides as reached the potatoes as migrants (compare centre 18 below).

(v) *Centres* 10 *and* 12, with 67 and 176 aphides/100 leaves respectively. These two centres were near to villages some 9 miles from Cupar, which town provided meteorological data showing that 16 days in June had a maximum temperature above 65° F. and had a wind velocity at 9 a.m. of less than 5 m.p.h., whilst on 11 of these days the relative humidity at

9 a.m. was below 75 %. It is unfortunate that no count was taken under market-garden conditions in the immediate vicinity of Cupar.

Centres of low aphis infestation

(i) *Centres* 22–26, with aphis counts not exceeding 9/100 leaves. In the Montrose district, although market gardens were not inspected, the gardens near most of the farms were examined and supported the comparatively low aphis counts made at centres 22, 23, 24, 25 and 26. Meteorological data from Montrose showed that only on 6 days in June did the maximum temperature exceed 65° F. and there were only three occasions when the relative humidity at 9 a.m. fell below 75 %, whilst no single day had a wind velocity at 9 a.m. below 5 m.p.h.

(ii) *Centres* 33 *and* 34, with 0 aphides/100 leaves. The meteorological data from Banff indicated that, whereas the maximum temperature exceeded 65° F. on 13 days in June, only on 3 of these was the wind velocity at 9 a.m. below 5 m.p.h., and there were only 7 days when the relative humidity was below 75 %.

(iii) *Centres* 39–43, with not more than 8 aphides/100 leaves. The nearest source of meteorological data was at Fortrose, and clearly showed the high humidities prevailing in this area. Not a single day in June had a relative humidity at 9 a.m. below 75 % although the temperatures were favourable for flight on 13 days. Nine days in the vicinity of Fortrose had wind velocities below 5 m.p.h. at 9 a.m., but it is highly probable that this number would be less on the exposed peninsular conditions of Portmahomach where centres 40–43 were located.

(iv) *Centre* 18, with 29 aphides/100 leaves, deserves separate mention in that it was located within 2 miles of centre 19 (cf. (iv) above) where the conditions as regards temperature and humidity favoured flight, but a sufficiently low wind velocity occurred only on 5 days. This latter fact may be regarded as sufficient to explain the low count at centre 18 in contrast to the high count taken at centre 19.

Conclusions

It is desirable that studies on the effect of meteorological factors upon migration of aphides under Scottish conditions should be continued and amplified. Nevertheless, the results obtained during the present survey when viewed in the light of field observations at the College Farm, Bangor, in north Wales, lend considerable support to the conclusion that migration of aphides does not attain appreciable intensity unless the

temperature exceeds 65° F., the wind velocities remain below 5 m.p.h., and the relative humidities do not exceed 75 %.

The consistent increase in numbers of aphides in fields to the *east* of urban areas, as compared with that found to the *west*, strongly suggests that the winged forms migrated during periods of drier, southerly and west breezes from the land. In this case, therefore, the main migration would be in the opposite direction from that obtaining in north Wales, and would indicate that migration follows the direction of the drier breezes whether these blow from the west, as in eastern Scotland, or from the east as is the case in north Wales.

DISCUSSION

Aphis population

It should be remembered that the two years, 1933 and 1934, previous to the present survey were "peak" years for aphis infestation, at least in north Wales, and this fact must necessarily limit the extent to which the results of the survey can be used as a basis for generalizing. Yet, the important fact remains that, even by taking random samples of 100 lower leaves, no centre was found where the potato crops were absolutely free from aphides, including *Myzus persicae* the vector of so many virus diseases. After some years' experience of species of aphides attacking potatoes, the writer doubts whether there is any locality in Great Britain in which potatoes are grown on any scale where aphides could not be found during the month of July. This opinion admittedly requires much further work before it can be fully substantiated, and it would be of real interest in this connexion if potato crops could be examined in such isolated areas as the Outer Hebrides during the month of July.

The role of the winter host plants in the development of the aphis population has been shown by this survey to be of importance in eastern Scotland, as has already been proved to be the case in north Wales. There is also an indication that, in Scotland, centres to the east of towns may be more heavily infested than those on the western side. This, it is suggested, is to be correlated with the fact (ascertained from meteorological records) that the light, drier breezes during June came, overland, from the west.

The survey from the seed-potato production aspect

That there are districts where aphis population on potatoes is comparatively small is as apparent from these studies in eastern Scotland as it is in north Wales. Further, there is ample evidence, from the viewpoint

of the dissemination of virus diseases, that "stock" seed of excellent health has been grown for years in such districts of low aphis infestation. Thus, in the light of these facts, it becomes unnecessary to search for large areas which are entirely free from aphides for the production of such special nursery stocks of exceptionally healthy seed potatoes. Greater advantage, however, might be taken of districts with low aphis infestation, with their comparative freedom from the potential danger of insect vectors, in producing special stocks which could be used to replenish other seed-producing centres, since these may, for economic or other reasons, have to extend into districts with a moderately heavy aphis infestation. On a small scale such a precaution is taken by this College under the scheme for producing seed potatoes in north Wales and, even in Scotland, it would be of value in ensuring that, during seasons of abnormal aphis infestation such as 1933 and 1934, there would be sufficient acreage of nursery stocks for later distribution. In this connexion it may be stressed that low aphis infestation is not of necessity associated with high altitudes, and an extension of seed-potato growing in selected low-lying wind-swept districts with high humidities is worthy of consideration. Further, it may be noted that districts even with low rainfall but with frequent sea mists will provide the degree of humidity unfavourable for aphis migration. In north Wales most of the successful seed-producing centres are low-lying and coastal.

The fact of the existence of such heavy aphis infestations as 901/100 leaves as far north as Morayshire stresses the need for insistence upon greater isolation of stocks grown for seed, even T.S. (H) stocks, than is afforded by a two-drill width of separation from potatoes with appreciable virus infection. Clearly, with such a population of aphides there is serious risk, since it has recently been proved that aphides find, and carry disease to, even single isolated potato plants from distances of at least a quarter of a mile (Davies & Whitehead, 1938). The use of swedes as the separating crop between potatoes in Scotland is also obviously open to criticism since this winter host supplies the means of over-wintering for the vector *Myzus persicae*. Indeed, the survey provides ample evidence to indicate the need for special precautions when seed-potato crops are grown in the immediate vicinity of market gardens and towns since, in addition to the probable presence in these districts of potato stocks of uncertain health, there is now the further evidence of higher aphis populations in such areas.

Finally, the survey has provided evidence of the considerable scope for entomological studies on insect vectors under Scottish conditions, and

it is hoped that the results of this survey will add a stimulus for some such investigation in the near future.

SUMMARY

1. This communication is the substance of a report on a survey of the aphis population in certain districts of Scotland carried out from 24 July to 4 August 1936.

2. Forty-three centres in eastern Scotland, from Midlothian in the south to Ross-shire in the north, were examined by the standard method for determining the aphis "index" figure.

3. The varying figures obtained have been analysed with a view to establishing any relation there might be with (a) proximity to winter host plants, and (b) meteorological conditions such as temperature, humidity and wind velocity.

4. The results have been summarized in a discussion in which are indicated the special precautions necessary to ensure health in seed-potato stocks.

REFERENCES

CURRIE, J. F. (1933). The production of high-grade seed potatoes in north Wales. *J. Minist. Agric.* **40,** 316.

DAVIES, W. M. (1932). Ecological studies on aphides infesting the potato crop. *Bull. ent. Res.* **23,** 525.

—— (1934). Studies on aphides infesting the potato crop. II. Aphis survey: its bearing upon the selection of districts for seed-potato production. *Ann. appl. Biol.* **21,** 283.

DAVIES, W. M. (1935a). Studies on aphides infesting the potato crop. III. Effect of variation in relative humidity on the flight of *Myzus persicae* Sulz. *Ann. appl. Biol.* **22**, 106.

—— (1935b). A water-power mechanical insect trap. *Bull. ent. Res.* **26**, 553.

—— (1936). Studies on aphides infesting the potato crop. V. Laboratory experiments on the effect of wind velocity on the flight of *Myzus persicae* Sulz. *Ann. appl. Biol.* **23**, 401.

DAVIES, W. M. & WHITEHEAD, T. (1935). Studies on aphides infesting the potato crop. IV. Notes on the migration and condition of alate *Myzus persicae* Sulz. *Ann. appl. Biol.* **22**, 549.

DAVIES (the late), W. M. & WHITEHEAD, T. (1938). Studies on aphides infesting the potato crop. VI. Aphis infestation of isolated plants. *Ann. appl. Biol.* **25**, 122.

ELZE, D. L. (1927). De Verspreding van Virusziekten van de Aardappel door Insekten. *Meded. LandbHoogesch., Wageningen.*

SCHULTZ, E. S. & FOLSOM, D. (1925). Infection and dissemination experiments with degeneration diseases of potatoes; observations in 1923. *J. agric. Res.* **30**, 493.

SMITH, K. M. (1929). Studies on plant virus diseases. V. Insect transmission of potato leaf-roll. *Ann. appl. Biol.* **16**, 209.

—— (1931). Studies on plant virus diseases. IX. Some further experiments on the insect transmission of potato leaf-roll. *Ann. appl. Biol.* **18**, 141.

WHITEHEAD, T., CURRIE, J. F. & DAVIES, W. M. (1932). Virus diseases in relation to commercial seed-potato production. *Ann. appl. Biol.* **19**, 529.

CRYPTORRHYNCHUS LAPATHI L. IN RELATION TO THE WATERMARK DISEASE OF THE CRICKET-BAT WILLOW

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THE poplar and willow borer or weevil, *Cryptorrhynchus lapathi* L., is well known as a pest of willows in Europe, and sometime prior to 1887 was introduced into the United States, where it has become established. The weevil has been described by numerous authors, and is discussed in most works on forest entomology including those of Gillanders (1912), Doane *et al.* (1936) and Chrystal (1937). The most complete account of its biology is given by Matheson (1917). The weevil is probably widely distributed in Great Britain although somewhat local, and its range of host plants is fairly wide, including various species of alder, willow, poplar and birch.

The eggs are laid in the bark of branches of two or more years of age in cavities excavated by the proboscis. The young larva at first feeds exclusively in the bark and the cambium. Later, when nearly full grown, the larva leaves the cambium and burrows into the wood. In the smaller branches the larval channel often lies in the central pith region. Frass consisting of small particles of wood is forced from the larval chamber, and is very conspicuous on infested branches. The pupal chamber is formed at the upper end of the larval burrow, which may be several inches in length and is packed with frass. The fully fed larva pupates in this chamber, and the adult on emergence cuts its way through the frass to the exterior.

The length of the life cycle apparently varies greatly. There may be a complete generation every year, or the total life cycle may extend over a period of 2 years. Adults emerging in the late summer probably do not leave the branch, but hibernate within the pupal chamber until the following spring. On leaving the pupal cells in the spring the weevils begin to feed on the young green shoots. They are voracious feeders, a single weevil kept under observation making as many as sixteen feeding punctures in a shoot in 24 hr. There seems to be a distinct preference for the 1-year-old shoots, although feeding also occurs on woody shoots of greater age. Attacked shoots usually bend over in a characteristic way or

are broken by the wind. When disturbed the weevils feign death and drop to the ground, and on being handled emit a squeaking sound.

The weevil is usually responsible for some damage in most areas where osiers are grown, but few records appear to exist of the infestation of the cricket-bat willow, *Salix alba* var. *caerulea* Smith. Dr H. F. Barnes informed me of the occurrence of the weevil in considerable numbers at Batford, near Harpenden, Herts, on *S. viminalis* L., and, although bat willows were situated nearby, they did not appear to be infested. Severe infestations of bat willows were observed in Kent in 1928 and 1929, and a piece of infested bat willow was received from Lyonshall, Hereford, in 1937. Haines (1937) records that at Linwood, near Ringwood, Hants, the weevil is extending its attacks from *S. viminalis* to the bat willow. On visiting this locality, the infestations were found to be severe, the larvae tunnelling in the main stems of many young trees. *S. caprea* L. was also found to be infested, but poplars and alders were unattacked.

S. alba var. *caerulea* yields the timber used in the manufacture of cricket bats. Trees are grown on an extensive scale in many parts of East Anglia, especially Essex and Hertfordshire, although in recent years the tree has been planted elsewhere. The bat willow is subject to the disease known as "watermark", caused by *Bacterium salicis* (Day), emend. Dowson (1937), which renders the wood useless for bat manufacture. *B. salicis* is apparently restricted to the wood of the tree, and insects, which feed upon or inhabit the wood during a part of their life cycle, are suspected of being involved in the transmission of the disease. In the case of *Cryptorrhynchus lapathi* it is the feeding habits of the adult weevil which are of chief significance as regards disease transmission, the species apparently being well adapted for the mechanical introduction of *Bacterium salicis* into the wood.

A very similar watermark disease of willows other than *Salix alba* var. *caerulea* was investigated in Holland by Lindeijer (1932). She ascribed the disease to *Pseudomonas saliciperda* Lindeijer, and stated that this disease must be considered identical with the watermark disease of the bat willow in England. She also showed that in Holland *Cryptorrhynchus lapathi* is closely associated with and is capable of transmitting the disease. Two infection experiments were made. In one of these three weevils were allowed to walk for several minutes on a pure culture of *Pseudomonas saliciperda* and then transferred to healthy branches of *Salix alba*. No infection resulted in this case. In the second experiment three weevils were allowed to feed on diseased wood and were then transferred to a healthy branch. Infection occurred in this experiment,

although *Pseudomonas saliciperda* was not isolated from the diseased wood. From this single positive experiment, Lindeijer concluded that the weevil is capable of transmitting the disease.

An attempt was made to repeat Lindeijer's experiments in connexion with the watermark disease of the bat willow. In 1936 nine weevils were used in three types of experiments. Weevils were allowed to feed on diseased shoots, or their probosces were smeared with the bacterial exudate from a watermarked shoot or with a culture of *Bacterium salicis*. They were then transferred to the branches of two healthy bat willows and to a number of healthy pot plants, on which they fed voraciously. No symptoms of the disease were observable in these plants and trees during 1937 and 1938.

These infection experiments were repeated in 1937 using forty-six weevils altogether, the majority being allowed to feed on diseased shoots on watermarked trees for a number of days. They were then allowed to feed on healthy pot plants and on the branches of four healthy bat willows. No symptoms of disease have appeared in 1938.

CONCLUSION

Although *Cryptorrhynchus lapathi* has been found occasionally attacking the bat willow, it has not yet been associated with the watermark disease in the field. Infection experiments designed to show if the insect is capable of transmitting the disease have as yet given no positive results.

REFERENCES

CHRYSTAL, R. N. (1937). *Insects of the British Woodlands.* London and New York.
DOANE, R. W., VAN DYKE, E. C., CHAMBERLAIN, W. J. & BURKE, H. E. (1936). *Forest Insects.* London.
DOWSON, W. J. (1937). *Bacterium salicis* Day, the cause of the watermark disease of the cricket-bat willow. *Ann. appl. Biol.* 24, 5–28.
GILLANDERS, A. T. (1912). *Forest Entomology.* Edinburgh and London.
HAINES, F. H. (1937). *Cryptorrhynchus lapathi* L. on *Salix alba* var. *caerulea* Sm. *J. Soc. Brit. Ent.* 1, 194.
LINDEIJER, E. J. (1932). *De Backterie-Ziekte van den Wilg Veroorzaakt door* Pseudomonas saliciperda *n.sp.* Baarn: Hollandia-Drukkerij.
MATHESON, R. (1917). The poplar and willow borer. *Bull. Cornell agric. Exp. Sta.* no. 388.

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ENCHYTRAEID WORMS AND THE BACTERIA BED METHOD OF SEWAGE TREATMENT

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(With Plate IX and 4 Text-figures)

CONTENTS

PART I. FIELD OBSERVATIONS

INTRODUCTION

IT has been realized from the earliest days of sewage bacteria beds that the larger organisms present, such as worms and insect larvae, play a significant part in the economy of the bed by scouring the medium and preventing the bed from becoming choked (Buswell, 1928). Few investigations have been made, however, to assess the parts played by the various organisms in the fauna.

Parkinson & Bell (1919) showed the capacity of the Collembolan, *Achorutes viaticus* Tulb., to keep open experimental bacteria beds, and advocated the deliberate employment of this insect. Welch (1914) made

observations on the behaviour of the Enchytraeid worm, *Lumbricillus rutilus* Welch, but did not consider its scouring action. Much work has been done at the New Jersey Agricultural Experiment Stations to determine the meaning of the natural sloughing of the zoogloeal growth which covers the medium. The beds concerned have a thin surface growth of *Stigeoclonium* (Isokontae) and *Oscillatoria* (Cyanophyceae) which shows no quantitative fluctuations. The dominant macro-organisms are nematodes, though Oligochaetes (Pristina and Aeolosoma) are also present. These organisms are said to increase prior to "off-loading" and to decrease afterwards. It is considered that their wriggling movements might loosen the film causing the sloughing (Rudolfs, 1924).

More recently Lloyd (1935, 1937) has made observations of the bacteria beds in the Leeds neighbourhood, where the surface growth is ordinarily a highly resistant layer of *Phormidium* (Cyanophyceae) with the capacity to become continuous when scouring organisms do not hold it in check. He has suggested that apart from the physical forces, such as sun and wind which rupture and tear off fragments of the film, the most important controlling factor may be the abundant Enchytraeid worms. The investigations here described were undertaken on the beds at Knostrop, Leeds, to test this suggestion, and to study more completely the conditions in the bed affecting the relations of the flora and fauna, and so afford a better understanding of the changes which are known to take place.

For this purpose the behaviour and distribution of the worms have been studied by field and experimental methods, involving the use of model beds.

THE BACTERIA BEDS, THEIR FLORA AND FAUNA

A description of the bacteria beds

Bacteria beds vary in construction, the conditions of working, and the character of the sewage treated. Each of these factors may have an important bearing on the nature of the flora and fauna.

The Leeds beds are rectangular, 6 ft. deep and sunk below the surface. They contain pebbles composing the medium which are 2–3 in. across in the surface layer, becoming smaller in the lower layers, and still deeper, mixed with gravel. The liquid sewage is conveyed from the settling tanks to the bed and delivered as a sheet of water from travelling distributors of the Mill's type. Each machine supplies an area of bed 75 yd. long and 20 yd. wide, and it takes 10–15 min. to traverse this area. The liquid,

trickling slowly through the pebbles, drains into the bed effluent channels which carry it to the final settling tanks. When the sewage flow is of average volume the beds are worked 16–20 hr. per day, but longer or shorter resting periods, depending on the volume of sewage to be treated, may be given usually at night. Sometimes the beds may be rested for a few days, and then only the topmost few inches of the medium dry out.

Conditions in the bacteria bed

The beds form a peculiar and in some respects unique environment which has been described by Lloyd (1935). The outstanding features are the much reduced fluctuations in the temperature of the beds compared with those of the atmosphere, the constant saturation of the bed with moisture, and the great depth of the habitable zone compared with natural environments.

The temperature of the beds deserves special mention. It is relatively uniform from 6 in. downwards, probably due to the heat of vital activities taking place in the bed (Lloyd, 1935). 3–4 in. below the surface the temperature is much higher (2–4° C.) than that of the atmosphere in winter and correspondingly lower in summer. Frost is exceedingly rare below the surface of the bed.

The flora of the bacteria bed

The surface stones are covered by a growth of *Phormidium* (Cyanophyceae) often 2–3 mm. in thickness. It has pronounced seasonal fluctuations in character and quantity. In spring it becomes detached and washed away almost completely from the surface down into the bed. The stones beneath are covered by a slimy, jelly-like, zoogloeal growth of fungi and bacteria which contains numerous other micro-organisms, mainly Protozoa. This covering is also cast off in spring at the same time as the surface growth, the two processes being collectively termed the "off-loading" of the beds.

The fauna of the bacteria beds

Apart from the micro-organisms present, the fauna includes a number of Oligochaetes, and of these the Enchytraeid, *Lumbricillus lineatus* Mull., is the most important, but the Lumbricid, *Lumbricus rubellus* Hoff., is also abundant. The latter is confined to the deeper layers of the bed, and rarely found in the upper 6 in. However, the small immature worms of this species are frequently seen in the surface layers. The adults are most in evidence in the final settling tanks, reaching there

from the beds via the effluent channels. In spring they occur in such numbers as to cover the bottom of these tanks to a depth of several inches, where they putrefy, causing an unpleasant stench. Another Oligochaete belonging to the Naididae is also found in the effluent channels. This worm, which is essentially aquatic in habit, has not been found in the bed, but it may occur among the pebbles at the bottom of the bed.

The larvae of various insects form one of the most important groups, rivalling the Oligochaetes in abundance. The commonest are *Psychoda severini* Tonn. and *P. alternata* Say., *Metriocnemus longitarsus* Goet. and *M. hirticollis* Staeg., and *Spaniotoma minima* Mg.

Slugs are typical denizens, being rather numerous at Leeds, and a small snail, *Limnea glabra* Mull., is also found. Mites and spiders are common, the former occurring on dry patches of the pebbles. Nematodes may also be abundant in the surface growth and on the stones. All these organisms, with the exception of the Arachnids, are scourers of the medium, but relations between them may alter when food becomes scarce, and Lloyd (1937) suggests that this factor may be of importance in determining the variations which occur in the biological balance of the bed flora and fauna.

DESCRIPTION AND HABITS OF *LUMBRICILLUS LINEATUS* MULL.

Description

Probably the most important organism contributing to the changes occurring in the bed is the worm *Lumbricillus lineatus*. It is pink in colour due to the haemoglobin in the blood, of small size, averaging 15 mm. in length and 1·25 mm. in diameter. It has approximately fifty segments. The general organization of the worm is much simpler than that of the Lumbricids. Apart from its size the chief distinction from typical earthworms lies in the number of setae per bundle which in this species is generally four to seven as compared with the two present in earthworms. The setae are ∫ shaped, arranged fan-wise, and occur in four bundles to each segment, two lateral and two ventral.

Examination of the diagnostic features shows that they agree most closely with those given for *L. lineatus* Mull., and this title is retained. Considerable doubt exists in the determination of the species of *Lumbricillus*; according to Beddard (1895) the genus contains nine species, but Michaelsen (1900) describes fourteen. Welch, studying the Enchytraeidae in America, decided that of the European species, *Lumbricillus lineatus* Mull., *subterraneus* Vejd., *litoreus* Hesse, *verrucosus* Clap., and *agilis*

(author ?) were one and the same. Stephenson (1922), after discussing the variability in this genus and the need for care in dealing with it, comes to a similar conclusion.

Habits

The worms occur in fluctuating numbers in the surface ·growth, and exhibit marked seasonal migrations. They remain burrowed in the growth by day, but observations made throughout the night in August (1937) showed that during darkness they come on to the upper surface. They move actively away as the light increases and are not found exposed during the day, being strongly photofugic. Immediately below the topmost layer of pebbles the worms are found in large numbers roughly averaging 100,000–150,000/cu. ft., and persist in reduced numbers to the bottom of the bed where they are periodically washed out by the sewage. Their occurrence below the surface in discrete clumps of from twenty to 200 individuals is a characteristic feature. The centre or "nucleus" of each cluster consists of a piece of alga from the surface or of detritus. When the worms are cleaned of all foreign matter the tendency to cluster is much reduced. This pronounced thigmotactic response to alga and sludge has been remarked upon by Welch (1914) who carried out experiments demonstrating the fact. The clustering habit might be considered as a protection against desiccation, and Welch pointed out that resistance to desiccation was increased when the worms occurred in masses. But it has been observed on a number of occasions that when the beds become dry the worms, instead of remaining in clumps, generally separate showing that it is not adaptive in this respect. The larvae of the common flies inhabiting the beds are often found with the clusters of worms, but it is still uncertain whether they feed on the worms or on the particles.

The worms are not immersed in the sewage but only covered by a surface film, and there seems to be little doubt that they must use atmospheric oxygen for respiratory purposes in a similar manner to ordinary earthworms.

The cocoons of the worms are oval and measure approximately 1·1 by 0·75 mm. The walls are transparent and the white eggs can be seen inside distinctly. They are found embedded in the alga and also occur on the pebbles often very abundantly, one for instance, $2 \times 2 \times 1$ in., had forty-three cocoons attached. They are deposited in considerably larger numbers on pebbles with a rough surface, and groups of cocoons are found in the crevices. As already mentioned they are attached to the

substratum and can resist the pressure applied by a small paint brush to dislodge them. This fact is of importance since attachment prevents the cocoons from being washed out of the bed by the stream of liquid percolating through, and so enables the worm to maintain its numbers. It may be the limiting factor that keeps out other Enchytraeids, since the genus *Lumbricillus* is the only one of this family of Oligochaetes in which the cocoons are definitely stated to be attached to the substratum (Stephenson, 1930), but full details of the other genera are lacking. This factor is also of importance to the relative abundance of the different flies as pointed out by Lloyd (1937), and Graham (unpublished) working on these beds has shown that this is so.

FIELD AND LABORATORY METHODS

The area selected for detailed study

Observations during the spring off-loading (January–July 1937) carried out on two beds showed that the changes were similar, so attention was concentrated on one for the remaining 9 months (August–May 1937–8). It was early apparent that the wind break afforded by a channelled wall, 4½ ft. high, running in a north to south direction, and carrying the sewage, was making an appreciable difference to the temperature of part of the bed during the colder months. The following records for two beds indicate the extent of this influence:

Bed	Close to wall	2 ft. on east side	4 ft. on east side	Middle of bed
2	7·6° C.	8·1° C.	8·0° C.	7·0° C.
3	7·8° C'	8·0° C.	8·3° C.	7·0° C.

The prevailing wind is westerly, so that such temperature differences commonly prevail. Since a difference in the growth of the alga had been noted in the sheltered region the quantitative estimations have been carried out in duplicate for 1 year, for what are now termed the "sheltered" and "exposed" parts of the bed, the former being an area extending for 6 ft. on the east side of the wall, the latter, the remaining area of the bed.

Field observations and methods for quantitative estimations

In view of the presumed importance of the worms in the bed, any investigation of the changes occurring at the surface and in the depths of the bed must include estimations of the abundance of the worms in the surface growth and also their distribution and movements in the bed. Therefore, routine observations have been made twice weekly, including a general survey of the condition of the alga, and particularly the abundance and distribution of the worms in it. An approximate estimate of the number of worms in the upper 6 in. of medium was made, and for convenience classified as follows: very abundant, abundant, common, and scarce. An examination of the pebbles for cocoons and insect larvae was also carried out.

The weather at the time of observation was recorded, and during winter and summer extremes a sample series of bed temperatures was taken at shallow depths. Daily temperature records of the atmosphere, bed surface, and at a depth of 30 in.

together with a record of the solids in the bed effluent, have been supplied by the Manager of the Leeds Sewage Works.

To determine the quantity of worms in the surface growth, 20 g. of it were collected at random over the bed into a jar. For estimating the number of worms in the bed below the surface, depths of 12 and 30 in. were taken. A satisfactory method for these depths included the use of lidded iron pipes 4 in. in diameter, sunk into the bed to the respective levels. Simple, open, muslin bags containing 20 g. of scalded alga were let down the pipe and rested on the medium. These bags were changed every week. The worms moved readily into them and fed upon the alga.

Quantitative estimations of the alga were obtained by scraping 1 sq. ft. of medium once a week. A wooden frame enclosing this area was used for guidance. The condition of this alga was also noted.

The number of worms washed out of the bed in the effluent was estimated from material supplied by Mr J. F. Graham, who carried out weekly strainings of the effluent for 5 min. periods over 1 year.

Treatment of the alga collected for quantitative estimations

It was necessary to separate the worms from the collected alga for counting, and some difficulty was experienced in forcing them out. Immersion in warm water or deoxygenated water proved inadequate, but similar treatment with a 0·05% solution of copper sulphate caused the worms to emerge before they were killed. The efficiency of this method was tested by examining treated alga, and less than 1% of the total number of worms remained concealed. In practice 20 g. of the alga were immersed in this weak solution and left overnight. Then the worms were teased free of it and counted under water in shallow glass dishes $10 \times 7 \times 2$ in., standing on black cardboard, ruled by white lines into 2 in. squares. The contents of the muslin bags were similarly treated. If the catch was reckoned to be less than 1000 the entire number was counted, if greater the worms were spread evenly over the bottom of the dish and one-quarter of the area counted. The material collected by straining the bed effluent was examined fresh in the shallow dishes.

The surface growth scraped from 1 sq. ft. of medium was transferred to filter paper, dried in an oven and weighed.

The reliability of the methods

Some idea of the accuracy of the methods employed for sampling was essential. The number of worms in the surface growth was tested by taking duplicate samples, and the following figures give the number in two separate samples for both the "exposed" and "sheltered" areas during the winter months:

Exposed area		Sheltered area	
Sample 1	Sample 2	Sample 1	Sample 2
28	14	460	590
74	44	162	177
41	68	326	291
0	1	181	174
510	790	402	290
90	95	387	315
128	139	236	234
2	1	—	—
47	44	—	—
130	135	—	—

In summer the worms are much more abundant, and the numbers in four separate samples of alga, taken on two different occasions, were:

	Sample 1	Sample 2	Sample 3	Sample 4
6 Aug.	1750	1750	2000	1750
7 Aug.	1500	1750	2250	2000

The reliability of the method used for the 12 and 30 in. levels was tested by using two bags for one tube on four occasions. The numbers are given below:

₁ 1	Sample 2
2600	2350
1750	1400
1400	1150
2000	2000

The agreement shown is quite reasonable, and the average gives a fair estimate of worm abundance. The close similarity in the fluctuations for the "exposed" and "sheltered" areas, both in worm numbers and quantity of alga, indicates that the sampling is fair (see Text-fig. 1).

Some idea of the movement of the worms into the bags was obtained by having two bags in one pipe, one of which remained in position the whole week while the other was replaced each day. It was found that the number migrating into the bag from day to day fluctuated greatly but with a certain regularity in that a large entry generally preceded a small entry on the following day, and conversely. With one exception a constant ratio of 7 to 2 was found between the sum of the daily accumulations and the weekly accumulation:

Week	Week's aggregate of worms in bag changed daily	Number of worms in unchanged bag
1	4300	1400
2	2200	700
3	2300	600
4	7000	2200
5	2100	2000
6	3100	900
7	7700	2400

The evidence given by having two bags in one tube for the same period and the demonstration of a balance between the number of worms entering the bags and the number in the bed at that particular depth (ratio of 7 to 2) indicates that the method is representative. Any depletion of worms by their constant removal in the bags must be negligible because of their great abundance and their active migration locally.

DESCRIPTION OF THE SURFACE GROWTH OF PHORMIDIUM
AND ITS SEASONAL CHANGES

Description of the surface growth

The surface growth of alga consists of a thick covering of *Phormidium* (Cyanophyceae) which is distinguished from *Oscillatoria*, also found on the beds, by the much more compact nature of the growth due to agglutination of the filaments. Quick growth of these filaments accompanied by

their rapid formation enables a considerable stratum to form in a relatively short time.

A definite cycle of growth takes place from one sloughing to the next. The first stage in the recolonization of a clean pebble (Pl. IX, fig. 1) is the spreading of a thin layer of filaments from a number of centres which join up to form a continuous layer. This gradually becomes thicker (up to 2–3 mm.) and assumes a leathery condition (Pl. IX, fig. 2). The appearance of nodules, for the most part solid, has been commonly observed at this stage, and at these places the sheet ruptures and the edges curl away. The smooth layer may also be destroyed by weathering, particularly excessive drying-off and wind action, giving rise to ragged, curled edges. The sum total of these forces together with the depredations of insect larvae, worms and nematodes reduces the quantity of the growth (Pl. IX, fig. 4). One further important change takes place, particularly during the warmer months of the year. Part of the alga assumes what has been termed the "spongy" condition. In this state the growth loses its compact, leathery nature, becoming very soft with an irregular surface in contrast to its former smoothness (Pl. IX, fig. 3).

Seasonal changes of the surface growth

During the winter months in which these observations were commenced (January–March 1937) the surface growth remained leathery and generally firmly attached to the pebbles. The presence of incomplete patches with the edges curling away showed the effects of weathering and the activities of the scouring organisms. A few of the pebbles were clean or had a new growth forming. In March there was a slight decrease in quantity of the thick growth with an increase of clean pebbles.

Early in April a distinct change was observed, the alga had become extremely soft and spongy and riddled with worms. A few days later it was entirely spongy and lying loosely on the pebbles, a living mass of worms and impalpable debris. This condition prevailed until early in May, when it was apparent that a considerable reduction in quantity of growth had occurred. The reduction continued until, a few days later, the pebbles were either clean or had only small patches adhering. This change is called the off-loading of the surface growth and is characterized by the rapid onset of sponginess and rapid reduction in quantity. The worms also left the surface, since there was little to feed upon or to conceal them.

During this period of scarcity of *Phormidium*, a bright green covering of *Ulothrix* (Isokontae) and *Chlorella* (Isokontae) appeared on the pebbles.

An obvious new growth of *Phormidium* commenced at the end of May, rapidly replacing the other algae, and early in June a thin sheet was covering the medium. A thick continuous sheet was formed on most of the pebbles by the middle of this month. Throughout June and the first half of July the growth remained for the most part leathery, but was beginning to show the effects of weathering. In the latter part of this month, when the worms had again increased, areas of spongy alga coincident with worm aggregations were noticed. During the summer and autumn, and as late as November, the alga fluctuated continually between the spongy and leathery conditions, remaining bulky all the time. These changes sometimes took place rapidly, so that the pitted surface of the growth gave the impression of sponginess, whereas it was quite firm. The appearance of the spongy condition, without exception, coincided with the presence of large numbers of worms in the growth, and the leathery condition with their scarcity. The degree of dominance of one condition over the other seemed to be determined by the length of time the worms remained in the growth. One important point concerning the sponginess of the alga during the summer is that in a large number of cases it was of a more superficial nature, and the base of the sheets remained adherent to the substratum instead of lying loosely upon it. In the later weeks of October the alga had become completely spongy around the edges of the pebbles, and this was washed off leaving a thick central capping.

During the ensuing months (November–January 1937–8) the alga remained unchanged. The first indication of sponginess was seen in the third week of January, at the time when the worms first became abundant in the growth. The alga was almost entirely spongy and riddled with worms by the second week of February (2600 worms/sq. ft.). This early appearance of the worms *en masse* and the subsequent off-loading can be accounted for by the mild weather experienced in the early months of this particular year (1938) which precipitated events. During the next fortnight considerable reduction in quantity took place comparable with that in April of the previous year. (In the graph (Text-fig. 1) showing the numbers of worms in the alga, this rapid increase is masked by the low numbers which followed it.) The alga remained scarce until the end of the first week in March, when regrowth was so rapid that a fairly thick stratum was formed a fortnight later. During April the growth gradually became spongy again in certain areas where the worms had increased.

The growth in the "sheltered" area underwent similar changes but was consistently less in quantity, probably partly due to the shorter period of sunlight through interference by the wall.

The quantity of growth is shown in Text-fig. 1; for March 1938 two readings are given because two distinct phases occur in this month, namely, the end of the off-loading and the spreading of a new growth. It will be noted that the maximum dry weight/sq. ft. was 8·4 g. and occurred in August, while the minimum was 1·7 g. ("exposed" areas) occurring in May 1937.

<div align="center">

THE ABUNDANCE AND SEASONAL DISTRIBUTION OF THE
ENCHYTRAEID WORMS

Factors influencing the abundance of the worms

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The striking abundance of the worms indicates that these particular bacteria beds afford an especially favourable environment for them. A number of factors probably contribute to this: first, the medium is always covered by a film of sewage and, even when the beds are rested for a few days, only the upper few inches dry out, so that the worms are protected against desiccation. The temperature of the beds is more favourable than some of the natural habitats (Stephenson, 1930), with the result that in the winter months the vital activities of the worm, particularly breeding which occurs mainly from November to May, are much enhanced. Observations have shown that the production of cocoons, rate of development and viability are adversely affected by temperatures below 6° C., and in only 1 month in the period of 5 years has the mean monthly temperature in the bed fallen below this critical point.

The sewage continually brings a supply of food in the form of suspended solids, which is further augmented by the surface growth and fungal growths in the depths. There are no typically carnivorous organisms common in the beds likely to feed upon the worms, and predation in general seems to be at a low level. They are, however, exposed to the attacks of various birds, especially starlings and meadow pipits, when they occur in the surface growth.

The absence of any drastic chemical treatment of the sewage at Knostrop before it is conveyed on to the beds is also an important point affecting the survival and increase of the worms.

<div align="center">

Seasonal distribution of the worms

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The abundance of the worms in the surface growth, in the depths, and in the bed effluent has a seasonal variation. The fluctuations of the number in the surface growth during the winter months show a definite correlation with temperature changes, which is sometimes modified by

resting of the beds. From a study of the weekly variations and by means of experiment it has been shown that the worms migrate from cold. The distribution of large numbers of worms in columns of pebbles with a graded temperature was observed, and it was found that they moved downwards from the surface when this was chilled to 5–6° C.

Throughout the cold months the worms remain relatively scarce in the growth, but immediately the temperature rises in spring they migrate into it in very great numbers, and so long as the growth remains sufficiently thick are abundant until the temperature falls again. The worms are generally more abundant in the "sheltered" area in winter, which is almost certainly due to the higher temperature prevailing there. For only one short period in March 1937 did the reverse occur, and at this time an easterly wind was blowing equalizing the temperature of the two areas. The number of worms in the surface growth varies greatly in summer. This is not caused by temperature changes but is due to the bed drying off while it is being rested. It is less apparent in winter owing to the much decreased rate of drying.

Seasonal variations are also shown to occur at the 12 and 30 in. levels; here again the worms are scarce in winter becoming abundant during the spring, but they decline somewhat in summer when the worms are most numerous at the surface (Text-fig. 1). There can be no temperature control at these depths such as occurs at the surface, since the lowest average temperature is 9° C. which does not affect the worms adversely; in fact, this temperature at the surface in winter attracts the worms. The temperature fall in winter might reduce their activity and so alter the rate of entry into the bags of food, but this seems improbable, since observations of the worms at temperatures ranging from 3 to 25° C. have shown that it is only below 5–6° C. that activity is reduced appreciably. This agrees with Welch's work (1914) on the effect of temperature on worm activity. Further, the relative scarcity of the worms at these depths in summer must be accounted for.

A relative abundance of worms and cocoons in the bed effluent is confined to the spring months of the year. The temperature of the bed is uniform, as is shown by the fact that the temperatures of the tank and bed effluents are found to be very similar. This again discounts the presence of any temperature influence operating at the bottom of the bed.

It would appear from Text-fig. 1 or Table I that a considerable absolute reduction in worm population occurs in winter. That this is more apparent than real and due to the relative scarcity from the regions tested is shown by the fact that during the winter and early spring, whenever

the temperature allowed, the worms became abundant at once in the surface growth. This statement is further supported by the observations of their concentration in the upper 6 in. which showed that they remain abundant here throughout, only receding below 3–4 in. at very low temperatures.

Text-fig. 1 is a graphic picture of the relative distribution of the worms in the bed at various levels, on a depth scale during the period covered. The quantity of alga on the surface is also shown, together with the solids issuing in the final effluent. A temperature scale for the surface and the 30 in. level is shown below.
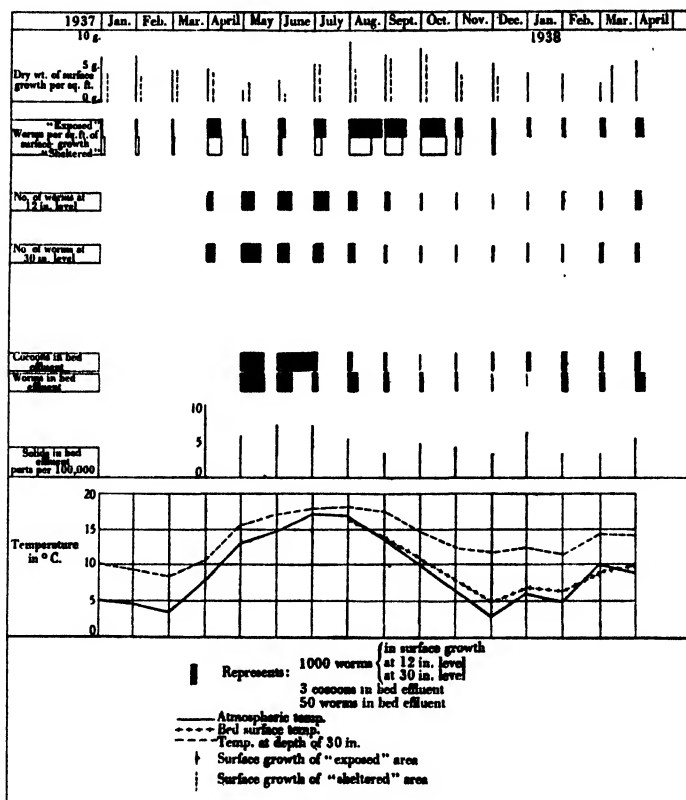
Text-fig. 1. The seasonal abundance and distribution of the worms in the bed on a depth scale, the quantity of surface growth, solids in bed effluent and temperatures, in monthly averages.

Table I. *Number of worms at the surface, 12 in. level, 30 in. level and in the bed effluent, the number of cocoons in the bed effluent, the quantity of surface growth and the bed temperatures. The figures for each month were obtained by averaging the weekly estimates*

| | No. of worms/sq. ft. in the surface growth | | No. of worms in bags of food | | No. in weekly strained sample of bed effluent | | Dry wt. in g. of surface growth/ sq. ft. | | Mean temperatures in ° C. | | |
	Exposed	Sheltered	12 in. level	30 in. level	Worms	Cocoons	Exposed	Sheltered	Atmosphere	Surface	30 in.
1937											
Jan.	300	600	—	—	—	—	6·3	3·8	4·9	—	10·1
Feb.	300	550	—	—	—	—	6·4	3·3	4·5	—	9·3
Mar.	200	400	—	—	—	—	4·6	4·5	2·5	—	8·5
Apr.	2500	2700	1000	1600	—	—	4·6	4·1	7·8	—	10·7
May	500	900	2500	4000	250	14·0	1·7	2·5	13·0	—	15·3
June	1000	400	2600	2400	160	21·0	2·9	1·2	14·75	—	17·0
July	2300	1400	3000	2000	60	3·3	5·4	5·4	17·75	—	18·0
Aug.	6600	4450	1450	1200	100	2·25	8·4	4·7	17·0	16·4	18·0
Sept.	4200	3400	800	400	20	1·0	6·5	6·0	13·3	13·9	17·8
Oct.	4950	5200	200	200	20	0·25	7·8	6·5	10·2	11·5	15·1
Nov.	1200	800	100	100	10	0·33	5·6	3·6	6·3	8·0	12·0
Dec.	400	300	50	100	5	0·75	5·5	3·3	3·5	4·6	9·0
1938											
Jan.	550	—	100	200	1	1·5	4·1	—	5·7	7·1	9·8
Feb.	600	—	200	200	30	3·25	3·9	—	5·2	6·4	9·0
Mar.	900	—	400	600	30	1·4	5·2	—	9·7	9·3	12·2
Apr.	1500	—	900	850	50	2·25	5·7	—	8·7	9·8	11·9

From these data it has been possible to construct a theory of the seasonal migrations of the worms. It is to be understood that the worms are scattered throughout the bed, but they concentrate at certain levels, and it is these concentrations which are referred to in the following account. During the winter months the worms remain scarce on the surface, the 12 and 30 in. levels, and also in the bed effluent, the bulk remaining in a layer from the surface to a depth of approximately 8 in. Immediately the temperature permits in spring they migrate into the surface growth in large numbers. Meanwhile a slight increase takes place in the depths. Soon after this movement into the alga the surface growth off-loads, and a new growth commences, the two processes occupying about 6 weeks, and during this time the worms are scarce at the surface due to the scarcity of food and concealment, but they increase greatly at 12 and 30 in.

The alga which has been washed from the surface, together with the material from the general off-loading of the medium, is slowly carried down the bed, followed by the worms and insect larvae which feed on it, causing an increase in their numbers lower in the bed. Some of the worms and larvae continue to migrate with the alga to the bottom of the bed and, as a result, a proportion of them are washed out in the bed effluent. This explanation receives strong support from observations of

Graham (unpublished) upon the relative abundance of larvae and pupae in the same bed effluent, contrasted with the number of larvae in the bed and the number of flies emerging from the bed. He finds that during the off-loading period considerably greater numbers of larvae and pupae occur in the bed effluent, and this is not solely due to increased numbers in the bed, but must be caused by a downward migration such as is postulated for the worms. In early summer, as soon as a considerable new growth has re-formed at the surface, the worms move up into this fresh source of food. It is also very probable that an upward migration from the depths, shown by the reduction of the worms at 12 and 30 in. (with no increase in the bed effluent), is accentuated by a distinct shortage
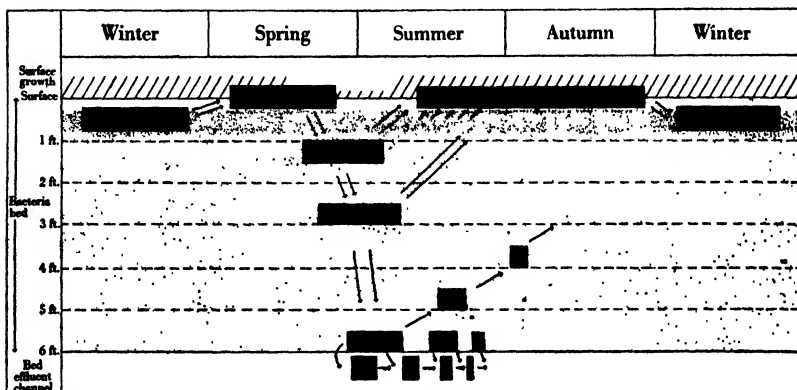
Text-fig. 2. A theoretical representation of the seasonal mass movements of the worms in the bed. General abundance in the bed is shown by density of stippling, and concentrations by rectangles.

of food in the depths at this season owing to the enormous increase of fly production. The concentration of worms remains at the surface in the growth during the remainder of the warm months, finally being driven below by the onset of cold weather. It does not reach a depth of 12 in. however, descending no more than necessary to avoid the cold.

The worms breed during the whole year, but this activity is greatest in the winter months. No influence of this factor on general abundance can be detected, and this contrasts remarkably with the behaviour of the various flies present (Lloyd, 1937). It is interesting to note that Welch (1914), studying a very similar case, found variation in abundance in the upper layers and bed effluent which would agree with this theory, although he does not offer any explanation. A diagrammatic representation of these mass movements is given in Text-fig. 2.

The effect of the worms on the character of the growth

It has been mentioned previously that the appearance of the spongy type of growth on the beds always coincided with the presence of large numbers of worms in it, and that the spring off-loading closely follows the spring migration of worms into the alga. Insect larvae are so scarce in the alga that their effect on surface growth can be ignored.

Examination of the growth showed that over the whole bed areas of spongy alga coincided with areas where the worms were very abundant, and that even in patches on single pebbles the same connexion was evident. Microscopic examinations of both the spongy and healthy types, collected from the same area, have been made on a number of occasions at different times of the year, including the off-loading periods. It was found that a greater accumulation of greenish brown granular worm faeces occurred in the spongy type of growth. There was no morphological difference in the filaments of the alga. An indication that the filaments of the healthy growth were slightly more vigorous than those of the spongy growth was given by the use of neutral red, which appeared to stain the protoplasm of the latter to some extent (Guilliermond, 1934). Protozoa were never present in quantity. Nematodes were fairly numerous in a few of the samples of both the healthy and spongy growth but showed no consistent correlation with any particular condition. This evidence suggests strongly that the worms are responsible for the degeneration of the growth.

To facilitate a study of the alga in the absence of the worms, pebbles were isolated in shallow pot trays, $10 \times 7 \times 2$ in., placed inside shallower larger trays, so that there was a moat $\frac{3}{4}$ in. wide between them. These trays were set into the bed, level with the surface, and the moat and inner tray filled with tank effluent as the distributing machines passed over them. Muslin bags containing copper sulphate crystals were placed in the moat twice weekly in winter and every day in spring. Three couples of such trays were used containing large, smooth pebbles with incipient growth on them, surrounded by smaller pebbles (P. IX, figs. 7, 8). The experiment was commenced in September 1937, and by the end of October a thick, smooth layer of alga had formed over each pebble. Later nodules appeared in the growth on some of the pebbles and these, together with the effects of weathering, resulted in the alga being torn away to some extent around the edges. The growth continued in this

healthy condition during the winter months. When the movement of worms into the surface growth took place towards the end of January and early in February, and the alga on the beds became generally spongy, that on the isolated pebbles remained unchanged, and during the subsequent off-loading it still retained its thick leathery growth. At this time it would have been impossible to choose five pebbles (the number in the trays) at random over the bed which had this type of covering, and this clearly indicates the significance of the test. As a final experiment the worms were introduced into one of the trays in March, and after 3 days the alga in this tray was becoming spongy where the worms had collected. These areas continued to spread (Pl. IX, fig. 6), and a fortnight after the introduction of the worms the alga was completely spongy. During this time the growth on the pebbles in the other trays did not alter.

This last evidence finally proves that the worms are responsible for the spongy character of the growth. The softness and sponginess is due to the worms feeding on it and its gradual conversion into faeces. In the advanced stage the worms seem to plough it, giving rise to the uneven, pitted surface.

The influence of the worms on the off-loading of the surface growth

That the off-loading of the algal growth occurs soon (14 days) after the spring invasion of worms is very significant, and there seems to be a definite connexion between the change to the spongy type of growth and the off-loading at this time. Further, no evidence of any other cause could be found. Drying of the bed surface causes no sponginess but a kind of peeling which results in discrete pieces of growth being dislodged and washed down amongst the pebbles, as later happened to the growth in one of the trays mentioned above; complete off-loading cannot, however, be accounted for in this manner. The absence of off-loading on the isolated pebbles at a time when it was taking place generally on the bed is important.

The main obstacle to this theory is that in summer when the worms are abundant in the growth and sponginess of the latter is common, a general off-loading does not occur. However, a reasonable explanation of this may be that during summer the algal growth tends to keep pace with the depredations of the worms. It is well known that the rate of growth of blue-green algae is increased by higher temperature and longer light (Hesse *et al.* 1937). Some idea of the high rate of growth on the beds in summer is given by the fact that when two selected areas were scraped

clean a new growth equivalent to 5·2 g. (dry weight)/sq. ft. re-formed in a month. Attachment to the substratum is often maintained during "summer sponginess", and it is probable that this is less firm in spring after the growth has withstood the weathering effects of winter, and so is more likely to off-load at this time. The autumn off-loading, which has been reported to occur sometimes, can be explained in a similar manner to the spring off-loading, the regeneration of the alga diminishing as the days shorten.

It is interesting to note that the severity of the off-loading in February 1938 was decidedly less than that of the previous year, and also that the attack of the worms was much less prolonged on this occasion due to a following cold spell of weather.

All this evidence clearly indicates a close connexion between temperature, season, and severity of worm attack as precipitating an off-loading of the surface growth.

PART II. THE INFLUENCE OF *LUMBRICILLUS LINEATUS* MULL. ON THE EFFICIENCY OF BACTERIA BEDS

INTRODUCTION

The scouring action of organisms in the bacteria bed has been emphasized in Part I, and also the special significance of the Enchytraeid worms in this connexion, particularly the relationship between them and the off-loading of the surface growth. The activity below the surface is not so obvious, and it was necessary to assess the part played there by means of laboratory tests. Model bacteria beds were therefore constructed, and the scouring capacity of the worms was studied under controlled conditions. Investigations of a similar kind but in relation to the Collembolan *Achorutes viaticus* Tulb. were carried out by Parkinson & Bell (1919). They found that a bed inoculated with this insect remained open and delivered effluent of a high quality with a good degree of nitrification, whilst a bed without a scouring organism became choked and useless.

DESCRIPTION OF APPARATUS

The experiment was carried out in a photographic dark room to reproduce as near as possible the conditions in the bed where only the upper surface is exposed to light.

Two glass cylinders, each 36 in. long and 3 in. in diameter, filled with marble chippings as a medium, functioned as bacteria beds and were supported side by side in a vertical position by a wooden frame (Text-fig. 3). The cylinders fitted circular depressions cut into a board upon which their lower ends rested. These depressions

were perforated by eight holes each of $\frac{1}{4}$ in. diameter, through which the liquid trickling down the bed could drain away into a sink over which the apparatus was fixed. The tank effluent was delivered on to the beds from two large aspirator bottles placed above the cylinders. The marble chippings used for the medium averaged about $\frac{1}{2}$ in. diameter and were packed evenly into the cylinders. The topmost 3 in. of the
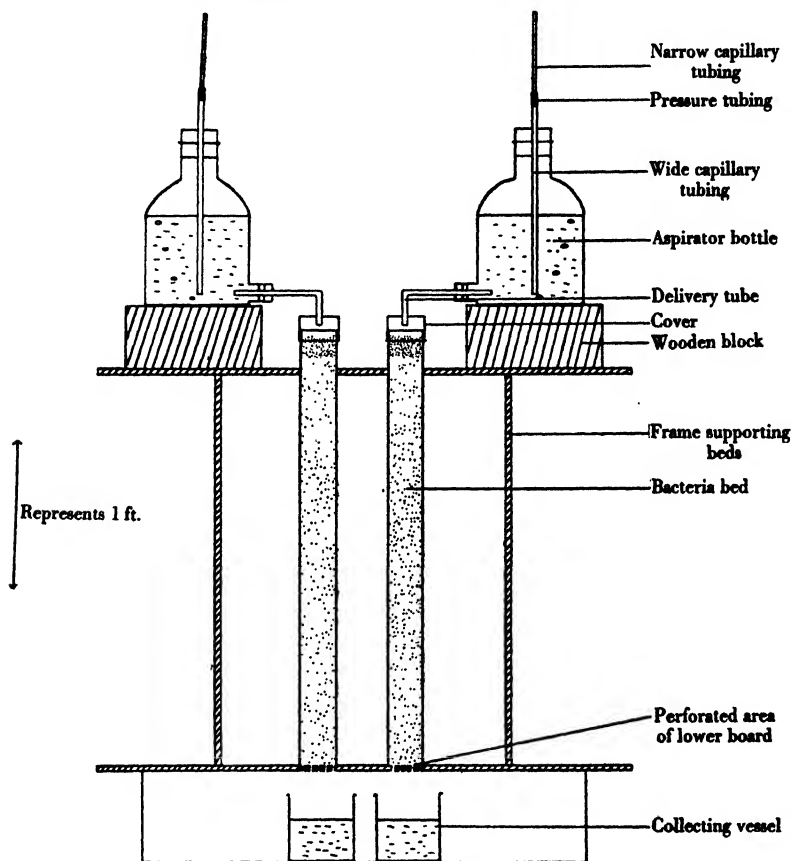
Text-fig. 3. Diagram of model bacteria beds.

medium consisted of chippings averaging $\frac{1}{4}$ in. diameter to spread the effluent evenly. Covers of bolting silk, supported in a cardboard frame into which the delivery tube from the bottle closely fitted, were kept over the tops of the glass cylinders. The portion of the sink used in the experiment was also screened off. These precautions were necessary to keep out the sewage flies. A barrier of bolting silk was placed between the two drainage areas of the beds after the introduction of the worms to prevent their migration from one cylinder to the other. It was possible to arrange a constant rate of

flow of any desired volume by constructing a hydrostatic head for each bottle. This entailed the use of capillary tubing with a bore 0·3 mm. in diameter, the rate of delivery being determined by the length of tubing used (Jenkins, 1933). This narrow capillary was attached to wider tubing which passed through the tightly fitting rubber bung of the bottle.

METHODS

The working of the model beds

The experiment was continued for a period of 23 weeks, from October 1937 until March 1938. Four and a half litres of tank effluent were delivered on to each bed daily, representing a rate of flow of 80,000 gal./acre/hr. The beds were worked for 12 hr. in the day, 6 days in the week and rested the remaining day. The tank effluent was heated up to 50° C. and cooled before use, as a precautionary measure against the presence of fly larvae and eggs. During the first 10 weeks crude sewage was used, but later it was strained through cotton-wool which removed the bulk of the coarser suspended solids and lightened the task of purification.

Chemical analysis of tank and bed effluents

Chemical analyses of the tank and bed effluents were carried out twice a week, and included determinations of the following according to the methods outlined in *The Standard Methods of Water and Sewage Analysis* (H.M.S.O. 1928):

(1) Free and saline ammonia, for both effluents.
(2) Albuminoid ammonia, for both effluents.
(3) Oxygen absorbed in 4 hr. from potassium permanganate, for both effluents.
(4) Nitrites in bed effluent.
(5) Nitrates in bed effluent.
(6) Suspended solids in bed effluent.

The samples were collected in beakers placed under each drainage area on the previous evening, but for the suspended solids a collecting vessel was used over the whole period between sampling. The condition of both beds, as far as could be determined, was noted from time to time.

They were worked for 5 weeks before the worms were introduced into bed A to make certain that both beds were functioning in a reasonably similar manner, and also to mature them before supplying the worms. On the eighteenth week worms were also added to bed B.

OBSERVATIONS OF THE BEDS

In both of the beds the medium was heavily coated with black deposits at the end of the third week, and these had become very heavy by the fourth week. During the next week approximately 1500 worms were added to bed A. They soon spread, and cocoons became common on the glass walls and medium. It was noticed by the tenth week (sixth after introduction) that the worms were reducing the areas of dense deposit. The following week an increase of solids began to appear in the effluent and soon became very heavy. This continued until about the

fifteenth week, the medium becoming gradually cleaner, until only slight deposits were present on the walls and marble chippings. All this time the solids continued to accumulate in the other bed until the medium could hardly be discerned, and black foul areas appeared. On the fourteenth week this bed was choked up and severe "ponding" occurred, i.e. the liquid was unable to drain through and collected on the surface. This meant that the bed was out of action and had to be rested for 5 days, but soon ponded again.

About this time there was a marked difference in the bed effluents, that from bed A was bright and clear as tap water, while that from bed B was milky and little different from the tank effluent.

On the eighteenth week worms were added to bed B to see if they would clean the medium as they had done in bed A. By the twentieth week they had spread uniformly in the bed, as far as could be determined, but, in the next few days, they all died in the bottom two-thirds of the cylinder. Worms were supplied on two further occasions, but each time they died, and on the last the whole bed became foul, so the attempt was abandoned. It seemed evident that this bed had become too septic for the worms to live in it for any length of time. Meanwhile they had continued to multiply in bed A. It was noticeable that after the introduction of the worms into bed B, it had become more porous so that no further sign of ponding had occurred.

RESULTS FROM THE CHEMICAL ANALYSIS OF THE TANK AND BED EFFLUENTS

The results, apart from the nitrites, nitrates and solids, are expressed as a percentage purification of the tank effluent by an average of the four analyses made over a period of 14 days (Text-fig. 4). Early analyses of the effluents from the two beds indicated that initial purification, as shown by the tests of free and saline ammonia and oxygen absorption, was slightly better in bed B, so the worms were introduced into the less efficient bed, namely, A. Two well-defined phases occurred in the bed containing the worms: first, the washing out of the solids from the bed after they had been loosened and broken up by the worms. This had an initial detrimental effect on the character of the bed effluent as shown by the estimations of albuminoid ammonia, and the oxygen absorbed, which was due to the solids present as analyses of the decanted liquid proved. The second phase covers the gradual recovery of this bed until a steady high efficiency was being maintained. The quantity of free and saline

ammonia is not affected by the amount of solids in the bed effluent, and this test shows a gradual increase in the standard of purification, especially in bed A. On the other hand, the control bed shows a steady,

Text-fig. 4. Results of the chemical analyses of the two bed effluents.
—— Bed A. – – – – Bed B.
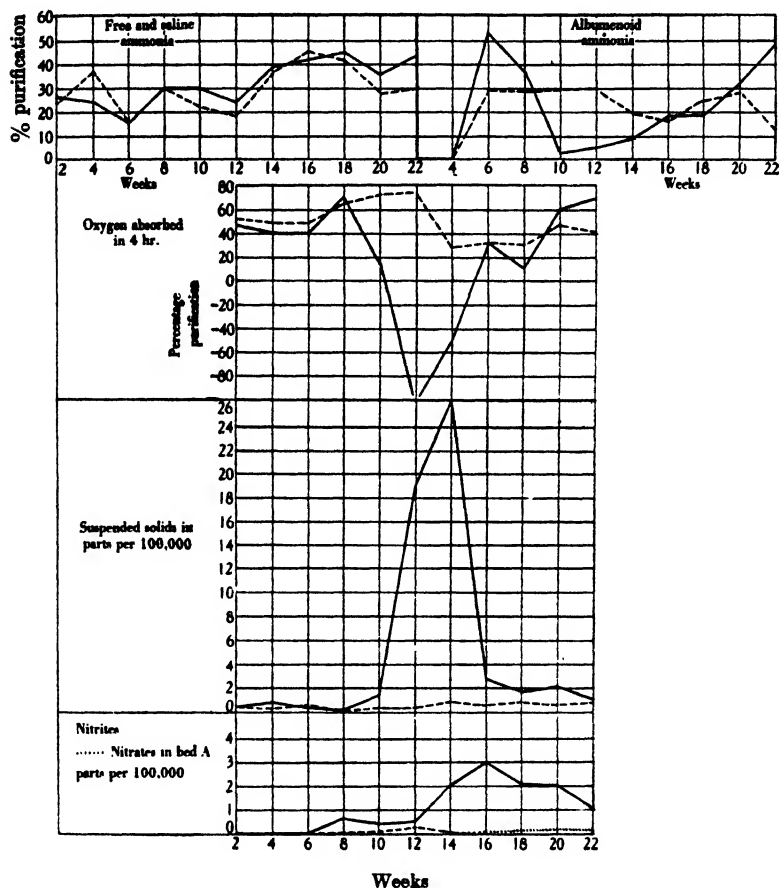
moderate efficiency over the first period, with a subsequent decrease, so that the degree of purification carried out in the two beds becomes very different. It seems probable that the presence of the dead worms in bed B in the last 2 weeks of the experiment increased the amount of albuminoid ammonia in the effluent from this bed.

The outstanding contrasts in the behaviour of the two beds are:

(1) The heavy off-loading of the solids in bed A caused by the worms, and their gradual accumulation in bed B, which was eventually choked.

(2) The active nitrification in bed A, in contrast to its virtual absence in bed B.

This last activity takes place only when the putrescible matter in sewage has been removed, and is often taken as a measure of the efficiency of a bed (Buswell, 1928).

The essential difference in the character of the bed effluents is further shown by the methylene blue test for stability. This was carried out over the last 8 weeks of the experiment. The effluent from bed A proved stable in thirteen out of sixteen tests, while that from bed B was putrescent in every case.

DISCUSSION OF PARTS I AND II

Observations recorded in this paper, together with those from bacteria beds in general, leave little doubt that the off-loading of the zoogloeal growth and accumulated solids, which occurs in the bed during the spring and sometimes in the autumn, is the result of biological activity.

Parkinson & Bell (1919) have shown that *Achorutes viaticus* (Collembola) is capable of causing it, and now the ability of *Lumbricillus lineatus* (Enchytraeidae) to bring about these changes has been demonstrated. Further, there appears to be no reason why other organisms of similar habits, living in the bed, should not behave in the same way. Actually the larvae of *Psychoda cinerea* Banks (*P. compar* Eat.) cleaned the medium of the model bacteria beds used in these experiments, when introduced accidentally, with the result that the preparation of the beds for the test on the activity of the worms had to be started afresh with the addition of certain precautionary measures mentioned above, such as the heating of the sewage to 50° C.

This spring phenomenon can be explained by the gradual stimulation of the bed fauna through the rise in temperature, but a more exact explanation can be given for the beds described in these experiments (see also Part I). Here the surface growth of *Phormidium* (Cyanophyceae) is slowly washed down into the bed in spring after being loosened, mainly through the greatly increased number of worms feeding on it. The worms and larvae continue to feed on this detached growth, following it into the depths of the bed, where they also attack and loosen the zoogloeal growth around the pebbles. In consequence, a general off-loading of the bed is precipitated. A further important feature of the activity of these

worms is that they also break up the growth and prevent its accumulation in the upper layers of the bed, which would otherwise become choked. The prevention of such choking in the bacteria bed still remains one of the outstanding problems of this widely used system of sewage treatment (Jenks, 1937). Hence the presence of the worms is essential if these beds are to remain open. Other forms, such as insect larvae and Nematodes, are also important in helping to break up the growth, but work mainly below the surface.

The fauna of bacteria beds varies with the sewage works according to local conditions and different organisms are dominant, but they perform this same function. At Huddersfield, for example, the surface growth and worms are absent, and here, presumably, the abundant larvae of *Psychoda alternata* Say. perform this cleansing of the bed (Golightly, unpublished). These beds are treated periodically with creosote to keep down the flies (Scouller & Goldthorpe, 1932), and this probably accounts for the absence of worms.

The impression given so far is that the action of these larger forms is solely beneficial. This is not strictly true as, occasionally, chemical treatment of the sewage is necessary to keep down the emerging flies. The need for this is due to the frequent imperfection of the biological balance which hinders seriously the work of purification. No information has been obtained upon the most desirable combination of organisms in the fauna for the production of the most efficient treatment of sewage by the bacteria bed method. Too little is known of the interrelationships of the bed faunas, and especially their ability to check or compete with one another under changing conditions. For instance, *P. alternata* and *Metriocnemus longitarsus* Goet. increase periodically and are a nuisance to nearby dwellings and to those employed on the beds, but their larvae are beneficial. Again, various worms and larvae are washed out of the beds into the final settling tanks, often in large numbers, where they decompose and cause a serious deterioration of the final effluent. The small Enchytraeid worms and the larvae are not so important in this respect as the bulky Lumbricid worms, and it seems probable that the harm done by these large earthworms is far in excess of any good they might accomplish, and hence they are undesirable.

Welch (1914) carried out experiments to determine the effect of *Lumbricillus rutilus* Welch (probably *L. lineatus*) on the putrescibility of sewage by enclosing the worms in stoppered bottles containing sewage, with a series of controls containing sewage alone. He found that the former became putrescible first, owing to the effects of the respiration of the

worms and the accumulation of their excreta, so that to this extent he considered the worms harmful. But it has been pointed out (Part I, p. 142) that the worms are not immersed in the sewage in the bacteria bed, but take their oxygen from the air circulating in the bed. Further, the experiment with the model beds suggests that the consumption of atmospheric oxygen, the presence of the worm's excreta, and the dead bodies, in a healthy bed, do not have any serious ill effect. Therefore, it is concluded that the Enchytraeid worms are on the whole beneficial, and of particular importance under the conditions obtaining at Leeds.

Only the mechanical effects of these macro-organisms have been thought important, and the involved biochemical changes taking place in the bacteria bed, with the ultimate production of nitrate from proteins, have been considered to be due to bacterial action alone. The introduction of *Achorutes viaticus* (Collembola) and *Lumbricillus lineatus* into experimental beds leads to increased nitrification of the effluent, and this may be due to an indirect influence as suggested by Dyson & Lloyd (1933), or, on the other hand, the chemical changes of metabolic activity in both Protozoa and Metazoa present may produce considerable effect in a more direct manner upon the efficiency of the treatment. Physiological studies of the organisms in the bed are essential therefore to a full appreciation of their influence.

SUMMARY OF PARTS I AND II

1. The flora and fauna of the bacteria bed of a sewage plant are described, and their interrelationships discussed.

2. A definite cycle in the character and quantity of the *Phormidium* (Cyanophyceae), the main constituent of the surface growth, is described, the most striking feature of the cycle being the spring off-loading.

3. The Enchytraeid worm, *Lumbricillus lineatus* Mull., is briefly described and its habits observed in relation to the conditions in the bed. The worms feed primarily on the growth of *Phormidium*, and consequently tend to cluster at the surface, when this is abundant and climatic conditions allow. Cold drives them slightly down, but the off-loading sends them deeply into the bed, when an increased proportion pass out with the effluent. Drying of the surface in summer sends them down in an erratic manner according to the degree of desiccation.

4. The cause of the off-loading of the surface growth in spring is its change from a leathery to a spongy condition. The worms are mainly responsible for this and, therefore, for the off-loading. The alga rapidly

re-forms (3–4 weeks) upon the pebbles, worms migrating into it again when it is sufficiently thick. The absence of an off-loading in summer when the growth is again spongy is attributed to the higher rate of regeneration owing to increased temperature and sunlight, which lessens the effect of the worm depredations.

5. The importance of *Lumbricillus lineatus* in keeping open a bacteria bed and helping to maintain its efficiency is proved by means of experimental beds. Worms introduced into an inefficient bed with solids rapidly accumulating cleared the medium and allowed active nitrification to commence. If the bed is very badly choked and septic, the worms may be unable to carry out this function. It is probable that all the larger scouring organisms abundant in the bed have this property to a greater or lesser extent.

6. The mechanism of the spring off-loading is discussed with special reference to the role of *L. lineatus* in the Leeds bacteria beds.

7. The importance of the maintenance of the biological balance by means of the most economic fauna is pointed out.

8. Finally, the need for more intensive physiological studies is stressed.

REFERENCES

BEDDARD, W. (1895). *Monograph of the Oligochaeta.* Oxford.
BUSWELL, A. M. (1928). *The Chemistry of Water and Sewage Treatment.* New York.
DYSON, J. B. & LLOYD, LL. (1933). Remarks on the flies breeding in the bacteria beds at the Knostrop Sewage Works, Leeds. *Proc. Inst. Sewage Purif.* Pt. 2.
GUILLIERMOND (1934). Le Vacuôme ou Système Vacuolaire. *Actualités Scientifiques.* Paris.
HESSE, R., ALLEE, W. C. & SCHMIDT, K. P. (1937). *Ecological Animal Geography.* London.
JENKINS, S. H. (1933). The design of experimental percolating filters. *Biochem. J.* 27, 240.
JENKS, H. N. (1937). The renaissance of the percolating filter. *Proc. Inst. Sewage Purif.* Pt. 1.

LLOYD, LL. (1935). The bacteria beds of sewage works as an environment for insects. *Proc. roy. Soc.* **10,** 34.

—— (1937). Observations on sewage flies; their seasonal incidence and abundance. *Proc. Inst. Sewage Purif.* Pt. 1.

MICHAELSEN, W. (1900). *Das Tierreich. Oligochaeta.* Berlin.

PARKINSON, W. H. & BELL, H. D. (1919). *Insect Life in Sewage Filters.* Sanit. Pub· Co.

RUDOLFS, W. (1924). Film removal studies on biology of sewage disposal. *Bull. N.J. agric. Exp. Sta.* no. 403.

SCOULLER, W. D. & GOLDTHORPE, H. H. (1932). Control of the sewage filter fly (*Psychoda*). *Publ. Hlth Congr.* 1932.

STEPHENSON, J. (1922). On some Scottish Oligochaeta with a note on encystment in a common fresh-water Oligochaete, *Lumbriculus variegatus* Mull. *Trans. roy. Soc. Edinb.* **53,** 2.

—— (1930). *The Oligochaeta.* Oxford.

WELCH, P. S. (1914). Studies on the Enchytraeidae of North America. *Bull. Ill. Lab. nat. Hist.* no. 10.

EXPLANATION OF PLATE IX

Figs. 1–4. Different types of surface growth.

Fig. 1. Clean pebble after off-loading.

Fig. 2. Firm healthy growth cut to indicate thickness.

Fig. 3. Spongy type of growth.

Fig. 4. Growth illustrating the effect of weathering and attacks of worms and other organisms.

Figs. 5–8. Pebbles isolated in the trays and the effects of the worms on the growth.

Fig. 5. Growth of control at end of experiment.

Fig. 6. Growth showing the effect of the worms on it.

Fig. 7. Growth on pebbles used as control, at commencement of experiment.

Fig. 8. Growth on pebbles before introduction of the worms.

Fig 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

ORDINARY MEETING of the Association of Applied Biologists held in the Imperial College of Science and Technology, London, on Friday, 7 October 1938. The morning session began at 11.45 a.m. in the Botany Lecture Theatre, the Chair being occupied by the President, Mr C. T. GIMINGHAM. The afternoon session began at 2.30 in the Botany Lecture Theatre, the Chair being taken by a Vice-President, Dr H. MARTIN.

Discussion on Fresh-water Biology and its Applications

The following papers were read:

I. Fresh-water biology and its applications: Introduction. By E. B. WORTHINGTON, M.A., Ph.D.

II. Physical and chemical aspects of organic production in lakes. By C. H. MORTIMER, B.Sc., Dr.Phil.

III. Algal physiology and organic production. By MARIE ROSEN-BERG, Dr.Phil.

IV. Some aspects of waterworks biology. By A. C. GARDINER, M.A.

I. FRESH-WATER BIOLOGY AND ITS APPLICATIONS: INTRODUCTION

BY E. B. WORTHINGTON, M.A., PH.D.

Director of the Freshwater Biological Association, Wray Castle, Ambleside, Westmorland

FRESH-WATER biology is of direct use to mankind in three different ways, in connexion with water supply, with fisheries, and with teaching. The last can be left out of account for present purposes, and of the other two it seems best to devote most attention in this discussion to water supply. The object of this introduction is to formulate the general problems, leaving others to give facts and take up specific questions.

In water supply and fisheries, just as in agriculture, the major biological problems are concerned with production of life, but research on the productivity of waters is still far behind that of the land. Between the two aspects of water supply and fisheries there is a big distinction—that water supply in general requires a reduction of productivity in order to avoid algal and other obnoxious growths, whereas fisheries, like agriculture, require an increase of productivity throughout all stages in the food cycle. The extent of the increase in productivity required is, however, dependent on the species of fish, because different fish, like crop plants, vary in their ecological requirements. The distinction in object mentioned above is not so great as it sounds, because

in order to increase or reduce productivity, we must know the factors by which it is controlled in nature. Moreover, the two aspects of reduction and increase may have to be considered together, because many natural waters carrying stocks of fish are used for supplying water, and many artificial reservoirs constructed for the storage of water are used for fishery purposes.

On the fishery side it is possible to make comparisons between the production of fish and of domestic animals or crops in terms of weight per unit area (see Dr Mortimer's paper). The conclusion is that, whereas production from fresh-water cannot compete with that from good agricultural land, it may do so with some types of poor forest or grazing land, so that under certain circumstances it may actually pay to convert land into water. Here there are two important considerations, (1) that land which can be converted into water most easily is usually waterlogged and of little use agriculturally, and (2) the sporting aspect of fresh-water fisheries is important: for example, the economic value of a trout lake depends on the amount of money which changes hands as a result of angling, and this sum is usually several times the market value of the fish caught. There may be opportunities in the future for increasing the production of water in this country up to the optimum for different kinds of fish, by manuring, a branch of biology which has been applied with great success on the continent in relation to the carp. The salmon, which provides the most valuable of Britain's fresh-water fisheries, from both netting and sporting points of view, presents many special problems which fall outside the scope of this discussion.

On the side of water supply, applications of biology are wanted mainly in connexion with the storage of surface waters rather than the derivation of underground supplies. The field for future work has been cleared to some extent by a short article by Prof. W. H. Pearsall in the *Annual Report of the Freshwater Biological Association for 1936*, and some of his conclusions may be quoted. The underlying principle is that results obtained from the study of natural waters, particularly of lakes, are applicable with modifications to reservoirs. Every environment is subject to change in its natural conditions, and in water this change is in general towards greater production of life. A lake or a river in a given set of geological and climatic conditions is subject to erosive forces. Sediment accumulates from the earliest days in the case of lakes, in the later stages of erosion in the case of rivers, and this sediment alters the chemical content of the water, and in shallow areas gives a hold for rooted vegetation. The accumulation and decay of dead animals and plants alters the composition of the deposits, the general tendency being towards an increase of organic matter. Thus any group of lakes or rivers can be arranged in a series which represents in broad outline the stages through which each will pass during its evolutionary history. It has been shown that in shallow water the larger plants, littoral algae, and bottom fauna vary in kind and number with these stages of development of bottom deposits. The same influences affect the free-swimming organisms, particularly the fish and insects. In a deep lake or reservoir, where the littoral zone is unimportant, the production of life centres round the plankton in its relation to the water and deep deposits. As the deposits increase in organic matter, so also do the nitrogen and phosphorus reserves increase in the mud; these are capable of being delivered into the water, especially at times of annual turn-over, so that evolved lakes or reservoirs may become increasingly independent in regard to chemical substances, and no longer influenced to so large an extent by the character of the inflowing water.

Now, regarding the application of these principles to reservoirs, the accumulation of nitrogen and phosphorus is highly undesirable because it involves increased productivity of algae. The rate at which their accumulation progresses depends on the situation: thus some reservoirs which were formerly pure water lakes, such as Thirlmere, progress very slowly, and indeed have changed but little in the past 15,000 years or so since the ice age. But where land has been converted into water, as in many reservoirs in England, progress may be very much more rapid; there are in fact cases where production has increased greatly during 50 or 60 years in the north of England, while in the south of England the rate of silting and increased production may be still higher, so that reservoirs constructed at the beginning of this century are already giving serious trouble.

Obviously some means must be developed of maintaining the *status quo* in reservoirs, and Pearsall suggests three possibilities: (1) the removal of nitrogen, phosphorus, etc., from inflowing waters, (2) the removal of mud from the bottom, which is possible in the case of series of reservoirs, where one or more can be put out of supply, (3) annual cropping to remove an amount of organic matter equivalent to the annual gain. It is clear that in many cases it should be easier and more efficient to remove the crop in the form of fish rather than plants, plankton, mud or bacteria. Hence, the conclusion is reached that an increased crop of fish may actually reduce the likelihood of trouble from the smaller forms of life which clog filter beds or otherwise lead to difficulties in the provision of pure water.

The above paragraphs outline the fundamental problem in the application of fresh-water biology to water supply, but before it can be solved, it is necessary to answer, albeit approximately, such questions as—in a given lake, reservoir or river what is the total stock of living plants and animals, and, in order to avoid an increase in the stock, what is the annual crop which must be removed? Answers to such questions would solve many problems with which the water undertaker is confronted to-day. They would help him in the siting of new reservoirs, in estimating the extent of filter beds required, in controlling inflowing waters of different chemical content in order to keep algae at a minimum, in deciding from what levels to draw water at different times of day and during the year, and how far it is desirable to stock reservoirs with fish and to encourage angling. In some of these questions knowledge is now sufficient to be of direct value, as the subsequent papers will show.

II. PHYSICAL AND CHEMICAL ASPECTS OF ORGANIC PRODUCTION IN LAKES

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Introduction and definitions

FOR the purposes of this paper the *productivity* of a lake is defined as the organic matter produced by the planktonic algae (phytoplankton). Production by rooted shore plants may be neglected in a large body of water. Production in this narrow sense is distinct from that in which it is frequently employed, namely, the production of all forms of life including the heterotrophic animals and bacteria. Heterotrophic forms merely consume organic matter produced by plants.

The *potential productivity* is determined by physical and chemical factors. Of the former light and temperature are important; they will be discussed in more detail in the following paper, but for present purposes it may be assumed that in most lakes little photosynthesis takes place below 15 to 20 m. Of the inorganic elements necessary for plant growth nitrogen and phosphorus are normally only present in small quantities in the water, so the depletion of one or other of these *limiting substances* may hold up production. Potential production is therefore directly determined by the rate of supply of the substances in question. Seasonal changes in light and temperature impress a seasonal rhythm on the organic cycle in a lake. In winter, lake water is more or less equal in temperature from top to bottom, and currents produced by the wind keep it in complete circulation. This results in the distribution throughout all depths of dissolved oxygen taken in at the surface, and of the nutrient salts produced by bacterial decomposition of organic matter in the mud. During the summer the surface waters warm up and become less dense. The strength of the wind-produced currents is not sufficient to force this warm, light water down into the denser cold water, so the circulation becomes confined to an upper layer, the *epilimnion*. This lies above a lower, colder stagnating layer, the *hypolimnion*, and there is a zone of sharp temperature change between them, the *thermocline*. In most cases the thermocline is at such a depth (15–20 m.) that photosynthesis is practically confined to the epilimnion. The algae produced here eventually sink into the hypolimnion and decompose there, or in the mud with which it is in contact. Organic elements are thus removed from the upper producing layers during the summer, and only return into circulation again when the lake cools down and "turns over" in the winter. Organic production is, therefore, controlled by the amount of the limiting substances present in the epilimnion at the beginning of such a stagnation period.

The seasonal cycle in a natural lake

The cycle in Windermere may be said to start with diatom growth in the spring, and it reaches its maximum at the beginning of June (Fig. 1). This growth is checked (Fig. 2) by the gradual, and eventually complete depletion of one or more nutrient salts in the epilimnion, which by this time is beginning to form. Conditions in the epilimnion after such a diatom maximum are characterized by constant depletion of minimum substances and an increased content of dissolved organic matter. These conditions are favourable to, and support, a population of blue-green algae, which, however, remain at a far lower level of production than that attained by the diatoms. Only a part of the organic capital produced by the diatoms in the spring is utilized by succeeding forms, such as the zooplankton (Fig. 1), in the same year. A large part of the crop falls into the hypolimnion, out of circulation, for that year. *Asterionella* contains approximately 50% dry weight of silica. The amount of diatom production calculated from the silicate depletion curve (Fig. 2) is in excess of the diatom dry weight actually found in the surface water.

Decomposition, with resulting mineralization in the hypolimnion water, and in the mud, uses up a part or all of the oxygen layer and enriches the hypolimnion with nutrient salts which, however, do not become available to the upper photosynthesizing layers until the end of the stagnation period. This cycle of events—i.e. production, followed by consumption and mineralization—with the seasonal rhythm imposed by thermal stratification, is similar in many, but not all, lakes and reservoirs (see

Mr Gardiner's paper). Phosphate is more often a limiting substance than nitrate. Silicate may limit diatom production (Fig. 2). In all cases the potential production is controlled by the rate of supply of the limiting substances from the mud and from the drainage basin. As the rate of supply from the mud depends on the original supply from the drainage basin it is clear that geological and climatic factors ultimately control organic production in water. In waters receiving relatively large concentrations of
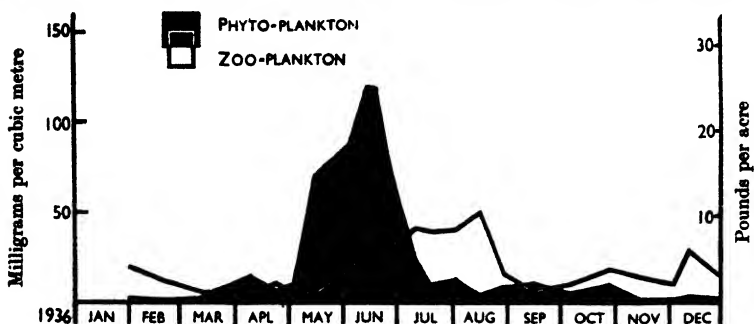
Fig. 1. Plankton production (dry weight) in Windermere.[1]
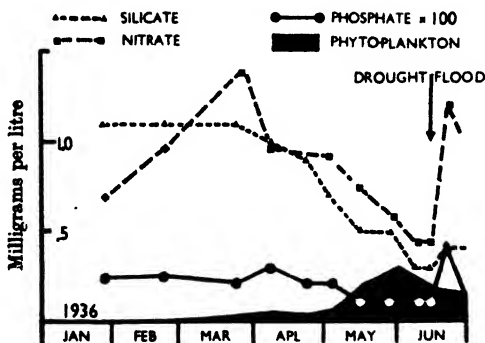
Fig. 2. Phytoplankton growth (dry weight) and nutrient salt depletion in Windermere surface water.

limiting substances (nitrogen and phosphorus) in the form of sewage effluents or drainage from agricultural soil, production is obviously increased. The influence of bases (calcium and magnesium) on organic production is also important. High organic production is rarely found in non-calcareous waters. The importance of climatic factors is seen in the effect of a flood after a drought in increasing the nutrient salt concentration (Fig. 2). Pearsall has shown that heavy floods in the early months of the year were followed by high spring diatom crops in Windermere. The converse was also true. It would appear that substances washed into the lake by these floods had increased potential production.

[1] Assuming same distribution throughout lake.

The measurement of production

For the study and scientific control of production some method of measurement is essential which will give results as crop weights per unit volume or area. The following are some methods which have been applied:

(1) Periodic sampling of the various forms of organic matter in the water can supply data for the calculation of the standing crop. For approximate measurements, and these are as much as one can usually hope to obtain, the zooplankton may be regarded as being the sample retained by a net of coarse silk (60 meshes to the inch), and the phytoplankton as being that which is retained by the finest net (180 meshes to the inch). This has been done in Fig. 1.

(2) Colorimetric or spectrographic estimation of the plant pigments, chlorophyll and others, extracted with various solvents, enable the estimation of plant material in a mixed sample to be made.

The above methods give a more or less accurate estimation of the standing crop at any one time. Until the algal physiologist can supply data of the reproduction and death rates of the various organisms under different conditions, the *gross production* of total weight or total numbers of organisms in one season can only be estimated indirectly by one of the following methods:

(3) An approximate assessment from standing crop figures on the estimation of fifty changes in stock per annum has been made for Lake Mendota. The gross production in one season was 10,700 lb./acre dry weight. The amount of zooplankton or fish allows one to gauge the general level of production. These animals have longer life cycles than the phytoplankton and more or less smooth over the large seasonal fluctuations which planktonic algae show.

(4) In special cases the extent and rate of depletion of a minimum substance enable the rate and extent of gross production to be measured with some accuracy. It is sometimes possible to prophesy when a certain substance will become depleted and stop growth. A case in point is the silicate depletion by diatoms in Fig. 2.

(5) It has been suggested by Strøm that the oxygen deficit arising in the hypolimnion at the end of a stagnation period is proportional to the amount of organic matter falling from the epilimnion and, therefore, to gross production during that period. If this deficit is corrected for reducing substances which appear if the oxygen is completely used up, and calculated per unit area of hypolimnion surface, the ratio of this figure to the main standing crop during the stagnation period is constant in lakes of widely differing productivity (Table I).

Table I[1]

Lake ...	Green Lake, Wisconsin	Lake Mendota, Wisconsin	Fureső, Denmark	Black Oak Lake, Wisconsin
Mean standing plankton crop in kg. dry matter/hectare	277	240	157	94
Ratio	100	89	62	36
Real oxygen deficit since turn-over in mg./cm.^{2} of hypolimnion surface	12·82	11·39	7·92	4·57
Ratio	100	87	57	34

[1] Compiled from data from Hutchinson, G. E., *Internat. Rev. Hydrobiol.* (1938), **36**.

Limitations of the present data, and possibilities of production control

The quantitative data of production biology outlined above are extremely meagre, but it is the lack of qualitative data which constitutes the most serious gap in our knowledge. Chemical work both in fresh waters and in the sea has clearly shown the effect of the depletion of minimum substances in limiting production, but it has little
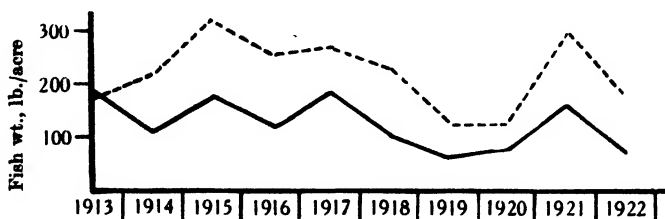
Fig. 3. Annual crop production in manured and unmanured carp ponds.[1]
– – – – Manured with phosphorus and potassium. ——— Unmanured controls.

to say about what starts the growth of an algal population. We are far from the ideal of being able to prophesy from a water analysis which algae will grow, and under what conditions they will appear. Cases in which all the minimum substances usually tested for in analyses are abundant, and no algal growth occurs, are not uncommon, whereas

Table II. *Annual crop weights in farming and fresh-water fisheries compared*

Type of farming	Food produced lb./acre/annum	Type of fishery	Food produced lb./acre/annum
I. Grazing:		Windermere*	Approx. 1·5
Meat the only product:		Alpine lakes†	Approx. 6·4
(a) From rich pasture	190	(average 10 lakes)	
(b) From poor pasture	20	*Carp ponds‡*	
II. Dairy farming:		Unmanured	100
Meat and milk joint products:		Manured	190
(a) Milk	2,000		
(b) Meat	45		
Total produce per acre	2,045		
III. Mixed arable farming:			
Various arable crops grown:			
(a) Wheat	1,950		
(b) Potatoes	17,920		
(c) Sugar beet	3,043 as sugar		

* Allen, K. R. Personal communication.
† Haempel, O. *Die Binnegewässer* (1930), **10**.
‡ Demoll, R. Teichdüngung. *Handb. Binnenfischerei Mitteleuropas* (1925), **4**.

in an apparently identical body of water a large outburst of growth may appear. It seems likely that there are yet more minimum substances stimulating algal growth of which we know nothing. The effect of winter floods on the Windermere diatom maxima, the productiveness of soil drainage in general, and the success of soil extract for

[1] Data from Demoll, R. Teichdüngung. *Handb. Binnenfischerei Mitteleuropas* (1925), **4**.

culturing all sorts of organisms, suggest that such unknown growth substances may be present in the soil. Although the lack of such essential data seriously limits the application of present knowledge to every case where the control of production is of practical importance, some attempts at chemical control have been very successful. Such a case is shown in Fig. 3. The addition of the limiting substances, phosphorus and potassium, increased the fish crop in all years above that of the controls, and the increase was proportionately greater in good years. A comparison of the crops produced in such artificial carp ponds, in natural lakes, and on agricultural land is of interest (Table II). Although meat production in most natural fisheries falls far below that produced in agriculture, intensive fish production on waste land, utilizing such waste products as sewage sludges and effluents might be an economic possibility.

III. ALGAL PHYSIOLOGY AND ORGANIC PRODUCTION

By Marie Rosenberg, Dr.Phil.

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In any problem connected with the balance of life or the production of organisms in water, the algae play a very important part. The general term "algae" covers a vast number of species of lower plants found in habitats differing widely in combinations of the factors important to plant life, namely light, temperature, and nutritive substances. Thus, some species live typically on melting snow at the edge of glaciers, others in hot springs; well characterized algal communities are known from waters with no detectable dissolved substances at one extreme, whereas practically saturated solutions of sodium chloride may show abundant development of Volvocales. A great variety of possible and actual combinations lies between these extremes in habitats, including many types of so-called fresh waters, with which we are concerned here.

These few examples give some idea of the range of habitats from which problems of ecology or physiology may be selected, all leading up to general questions concerning productivity.

We can distinguish, roughly speaking, three groups of processes going on in the water just as on land—production connected with the autotrophic plants, mainly the phytoplankton composed of algae, consumption connected with the animals, and reduction due to the work of bacteria. Chemical processes in the water have to be the principal basis for our understanding of the distribution of algae in space and in time. Comparative work in various countries has shown that it is possible to distinguish different types of waters, and to classify them as transition stages in an evolutionary series. In this connexion detailed work on a number of lakes has given interesting information as to the requirements of different groups of algae, and of their succession. Conclusions drawn from such observations must, however, be tentative, and must be checked by experiment. This second stage of causal analysis has not progressed very far, but it is of great importance to the theoretical and practical problems involved.

An annual periodicity in the numbers and species of phytoplankton has long been observed, maxima in spring and summer being the most striking general phenomena. The three groups of factors, light, temperature, and dissolved, nutritive substances, are being studied at the same time and correlated with the biological results. Production

of phytoplankton can only commence if and when the necessary conditions for cell division are fulfilled. The rate at which every single cell divides under a given set of conditions will be of utmost importance for the whole balance of chemicals and the supply of organic food for the heterotrophic organisms. Obviously, therefore, the conditions must be studied under which any given species or group of species attains its optimum rate of division.

The first way of approach, field work, has been followed for many years, with ever. improving technique in all branches concerned. For example, the penetration of light into water is now measured with photoelectric cells calibrated in physical units, thus expressing light in absolute values. This has been done in many cases and the results show interesting variations for different waters of the extension of the zone in which photosynthesis can take place. Thus, in some of the English lakes the same light intensity has been found in Bassenthwaite at a depth of 4 m., Windermere at 8 m., and Ennerdale at 21 m., readings having been taken in similar conditions. These figures, in conjunction with other data, express the productivity of the three lakes. Bassenthwaite was having at the time a huge diatom maximum, Windermere showed lower figures of plankton organisms, and the plankton of Ennerdale was very poor indeed. This example shows well the interrelationship of various factors. Although plenty of light was available in Ennerdale practically no plankton production was possible owing to a deficiency in dissolved substances. In Bassenthwaite, on the other hand, the mass production of plankton, developed owing to abundant dissolved substances, functioned as a screen and cut down the light for the underlying layers, thus limiting further production.

Considering temperature, the general principle holds that production increases with increasing temperature. Although, in some cases, optimum temperatures for the production of algae have been given, it is by no means clear that temperature is a factor of special importance for the distribution of phytoplankton. The importance of temperature for the distribution of chemicals, however, is clear, and certainly in this secondary way temperature changes are of great importance for the phytoplankton. This leads to the third and very important group of factors, the dissolved substances in the water.

In Dr Mortimer's paper the annual variation of several biologically important substances has been shown. As a demonstration of their importance for the algal flora a short outline of the periodicity of algae in a lake such as Windermere may be given. The general trend is a diatom maximum in the spring, followed by blue-green algae, then colonial green algae, Desmids, with another smaller diatom maximum in the autumn. The winter shows very low figures for all groups concerned, most forms disappearing from the plankton. The chemical changes in the water throughout the year justify the following conclusions. Diatoms, especially *Asterionella*, occur as dominants when the water is richest in nitrates, phosphates and silica. Volvocales and Desmids occur in largest numbers when nitrates and phosphates are low. Desmids particularly need a low calcium and nitrate/phosphate ratio for their maxima. Blue-green algae show their maximal growth when the organic matter dissolved in the water is high; they can grow rapidly in small quantities of nitrates and phosphates. These conclusions have been confirmed by comparative studies of several of the English lakes. Although geologically similar, the drainage areas differ mainly in their amount of agricultural land, thus supplying the lakes with very different amounts of bio-

logically important dissolved substances and silt. The lakes in question can be understood as successive stages of development from a poor or primitive to a rich and highly productive type. The main and first indicator for this change is the type of phytoplankton showing the same correlation with dissolved substances, as has been shown for the annual cycle in an individual lake. Tables I and II, taken from Pearsall, illustrate this:

Table I

Lake	Desmids	Green Colonial	*Dinobryon*	Desmids Colonial green	N/P
Ennerdale	76·0	17·0	—	4·5	9·6
Crummock	11·6	5·5	31·0	2·1	13·5
Wastwater	39·0	23·0	11·0	1·7	24·0
Derwentwater	7·5	3·5	39·0	2·1	13·8
Bassenthwaite	13·0	1·5	1·5	8·6	53·0
Lowes Water	10·5	1·5	4·0	7·0	49·1
Windermere	7·0	12·0	10·0	0·6	60·6
Ullswater	2·5	0·5	0·5	5·0	33·6
Esthwaite	2·0	5·5	1·5	0·36	54·5

Table II

	Albuminoid NH_3 in June	% of Myxophyceae in July
Bassenthwaite	0·010	0
Wastwater	0·026	0
Derwentwater	0·035	3
Ennerdale	0·038	7
Ullswater	0·045	2
Crummockwater	0·057	19
Windermere	0·061	26
Esthwaite	0·070	27
Windermere	0·075	46
Bassenthwaite	0·080 (July)	51 (August)
Lowes Water	0·082	61

Most of the examples mentioned imply the conception of a limiting factor of some kind. As in most other branches of biology it has become increasingly clear that it is impossible to consider one isolated factor alone. In fresh-water biology, especially in the case of the phytoplankton, it has become quite clear that we are always dealing with a complicated combination of factors and that the relative values of these may differ immensely in new combinations. We are always dealing with a certain *set* of conditions as a whole and must be careful to keep this in mind. One chemical substance, for instance, may have a most striking effect on division rate when present with another, and may fail entirely or even have an unfavourable or toxic effect when present in a different combination. The last word in all these cases must necessarily be left to the experiment, for this can clear up details otherwise inaccessible if field work alone is considered. Comparative experimental work on cultures under standard conditions has shown the usefulness of such controlled work, and it is hoped that the knowledge of the physiology of plankton algae will be furthered greatly by systematic experimental work—always of course in touch with conditions in nature.

IV. SOME ASPECTS OF WATERWORKS BIOLOGY

By A. C. GARDINER, M.A.

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DR MORTIMER has discussed the question of productivity in lakes and has used, in the main, data derived from Windermere. It is my task to show how this information can be applied to the reservoirs of the Metropolitan Water Board, which may be regarded as small lakes, receiving a river water rich in nutrient salts. In London, at any rate, the algal flora of the reservoirs is markedly different, both qualitatively and quantitatively, from that of the raw rivers from which they are supplied. As a rule, a very rich plankton develops seasonally in the reservoir. Concentrations of over 3 million individuals per litre of the diatom *Stephanodiscus astraea* have been recorded and even larger numbers of other forms. What this means in practice may be more readily appreciated if I say that in a large reservoir, holding some 6750 million gallons, the standing crop of the diatom *Fragilaria crotonensis* had, at the time of the spring maximum, an estimated dry weight of 110 tons. Although water was being drawn from near the bottom, where concentration of algae was less, it meant that the dry weight of *F. crotonensis* which had to be filtered off from this water was in the region of a ton a day.

In the case of the larger reservoirs it is now possible to forecast the probable duration of an algal outbreak. The method underlying such forecasting is based upon a knowledge of the factors controlling algal production. The date on which a particular growth is more likely than not to come to an end is arrived at from a study of the rate of algal increase and the rate of consumption of certain essential salts. The former is preferably obtained by counting, the latter by chemical analyses at intervals of not more than one week. The accuracy of the forecast depends, to some extent, upon stable weather conditions and necessitates a study of the salt content of the raw river water feeding the reservoir. The two dissolved substances, which have so far proved most useful in this respect, are phosphates and silicates. Briefly, if the rate of natural increase of the algae is such that the amount of phosphate and silicate is depleted more rapidly than fresh supplies are being brought in from the river, the time will come when concentrations of these substances fall to very low levels. When this occurs, the division rate slows up and finally the algae disappear from the plankton. The practical value of such a forecast lies in the fact that it enables one to decide whether remedial measures should be attempted or whether an intensive period of filter-bed cleaning will not suffice.

This method of forecasting is not possible in all cases. Where the ratio of volume of incoming water to volume impounded is high, the water in the reservoir is changing so rapidly that there is little chance of the salt-utilization rate of an algal population gaining on the rate of replenishment from the river. In other words, a high concentration of algae can be maintained with no marked fall in the salt content. What brings such an algal maximum to an end I have no idea. In the case of the diatoms, it may be that a relatively prolonged period of multiplication reduces the size of the resulting frustules to the minimum for the species, but for other classes of algae this does not apply. Save in a few cases, one hesitates to invoke temperature as a factor

of much moment: about the influence of light intensity our information is scanty, but I should doubt if it were decisive.

In such cases, remedial measures must be put in hand to deal with excessive growths. The two methods most commonly employed are the use of algicides, such as copper sulphate, or the application of a coagulant, like alum or ferric hydroxide. Neither is a sure remedy. The use of copper sulphate is attended by the risk of after-growths in the form of copper-resistant unicellular Chlorophyceae. Whilst alum has been found very successful in some cases, notably those of the diatoms *Stephanodiscus Hantzschii* and *St. astraea*, it has not proved of much use for *Fragilaria crotonensis*.

The last point in connexion with reservoirs is the difference between those in normal use and those which are allowed to stand. Any water undertaking which relies upon a number of relatively small storage basins must maintain a reserve above normal requirements, which reserves can be used in times of drought. This means that some reservoirs may be allowed to stand for a period of time which, if there be no drought, may be up to several years.

The most striking difference between the chemistry of a reservoir in normal use and one which has been allowed to stand is, perhaps, the alteration in the ammonia-nitrate ratio. In reservoirs in normal use, concentrations of nitrate are in the region of 0·17–0·22 part N/100,000, whilst those of ammonia are 0·002–0·007 part N/100,000. This relatively high concentration of nitrate is of some importance, because if we make the assumption that nitrogen as nitrate is the form most readily assimilated by diatoms, then it can be shown, from the ratio of nitrogen to phosphorus in the plant body, that nitrate can never be a limiting factor. In a standing reservoir, on the other hand, not only does the concentration of nitrate fall seasonally below that of ammonia, which for its part rises, but may become so low as now to be a limiting factor. In one reservoir, which has stood for a long time, concentration of nitrate this year has never exceeded 0·05 with a mean (January to October) of 0·009 part N/100,000. On many occasions the amount present was too low to be measured by the reduction method used. Since concentrations of phosphate and silicate rise when a reservoir is put out of normal use, the drop in nitrate, which I believe must result very largely from the activity of nitrate-reducing bacteria, assumes considerable importance. Had it remained normal, a really enormous algal production would have seemed inevitable.

Three cases have recently been investigated of reservoirs which have stood idle for some years. All are peculiar in showing a very heavy growth of the blue-green alga, *Oscillatoria rubescens*, during the colder months of the year. I am unable to say whether this growth is the direct result of the low nitrate content, which in turn, I believe to be correlated with standing idle; I can only say that it looks as if Cyanophyceae can flourish at very much lower levels of nitrate concentration than can diatoms. This is interesting, since analyses have shown that the percentage of nitrogen, expressed on an ash-free basis, is higher in planktonic Cyanophyceae than in diatoms. A consideration of the conditions prevailing during the prolonged period when *Osc. rubescens* was abundant in one of these standing reservoirs has led me very tentatively to suggest that this, and probably other blue-green algae, can fix atmospheric nitrogen. There is some evidence from the literature that certain Cyanophyceae can do this, but it is desired to emphasize that my own observations do not, as yet, entitle me to express any definite opinion.

The possibility that there is a direct connexion between concentration of nitrate

and production of Cyanophyceae raises a question of practical importance. Although *Oscillatoria* is readily removed by filtration, the disposal of sand wash-water presents considerable difficulties, since, on standing, it develops a bad smell and may be of an intense and lurid colour. To avoid a large production of the species, therefore, it might be advisable to advocate pumping river water into such reservoirs in order to maintain the nitrate concentration above that at which there is the risk of the appearance of *Osc. rubescens.*

Finally, I should like to draw attention to a subject which seems to me of some importance. The applied biologist is commonly called upon to deal with problems for which he has neither the answer nor the time in which to look for it. Some of these problems are capable of being tackled in Universities or in Research Institutions. It would undoubtedly be to our advantage if those in charge of what may be termed the more academic research laboratories could make a selection from such problems—many quite fundamental—which the applied biologist is expected to answer, as often as not on the telephone.

THE ASSOCIATION OF APPLIED BIOLOGISTS AND THE *ANNALS OF APPLIED BIOLOGY*— A RETROSPECT (1904–38)

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(With Plates X–XIII)

WITH the close of 1938 the *Annals of Applied Biology* completed its first 25 volumes, and the occasion appeared to be a suitable one on which to review progress in the Association and its journal.

I am indebted to various senior Members who read this account in typescript and gave me the benefit of their greater knowledge and experience.

I. THE ASSOCIATION OF APPLIED BIOLOGISTS

Prior to the year 1904 there was, in the United Kingdom, no scientific society or scientific journal devoted to applied biology. The active workers in the field were comparatively few, but the need was becoming evident for some central exchange and clearing house of ideas and research. In 1904, Mr Walter E. Collinge of Birmingham University raised the question of the founding of a society to serve these purposes and to advance the study of the applied aspects of biology. The idea was canvassed privately among a few interested people, and on the initiative of Mr W. E. Collinge and Mr F. V. Theobald, a meeting of workers interested in applied biology was called in the rooms of the Linnean Society of London on 8 November 1904.

There were present, Prof. G. S. Boulger, Mr W. E. Collinge, Dr H. T. Güssow, Mr E. M. Holmes, Mr A. E. Shipley and Mr Herbert Stone, with Mr F. V. Theobald in the Chair. "In a few introductory remarks the Chairman detailed the steps taken by Mr Collinge to found an Association of Economic Biologists. He hoped that it would welcome all investigators in Economic Biology, whether Agricultural, Medical, or Commercial. The interdependence of Biology, Agriculture, Medicine, and Commerce was apparent to all."

The Association of Economic Biologists was then inaugurated with the following Original Members: Prof. G. S. Boulger, Prof. A. H. R. Buller, Prof. G. H. Carpenter, Mr J. B. Carruthers, Mr W. E. Collinge, Prof. W. R. Fisher, Mr A. T. Gillanders, Mr E. M. Holmes, Mr D. Houston, Mr A. Howard, Mr A. D. Imms, Mr E. J. Lewis, Dr A. Stewart MacDougall, Dr F. H. A. Marshall, Mr H. Maxwell Lefroy, Mr Robert Newstead, Dr G. H. Pethybridge, Prof. Ronald Ross, Mr A. E. Shipley, Dr W. Somerville, Mr Herbert Stone, Mr Fraser Story, Mr F. V. Theobald, Mr R. Hedger Wallace, Mr Cecil Warburton, Mr F. C. Wilcocks.

which has elapsed since the foundation of the Association. The Inaugural Meeting was held on 8 November 1904, with twenty-seven Original Members,[1] and the number of Members on 21 December 1905 stood as follows:

Honorary Members		8
Ordinary Members		50
Associate Members		10
	Total	68 "

It was also noted in the Annual Report of the Council that "The total receipts up to December 25th were £33. 8s. 6d., including one life subscription of £7. 7s. 0d.; whilst the total expenditure for the same period amounts to £28. 17s. 11d., leaving a balance in the hands of the Honorary Treasurer of £4. 10s. 7d." Thirty Members and visitors attended the meeting and sixteen new Members and two Associates were elected. The President delivered the first Presidential Address on "Animal parasites and legislation", and nine other papers were contributed.

No meeting was arranged during 1906 but, in 1907, two meetings were held. The first of these took place in the Pathological Department of the University of Cambridge on 9, 10 and 11 January, and amongst the fourteen communications, one by Mr R. H. Biffen dealt with "Cereal breeding", and another by Mr E. S. Salmon was entitled "On the American gooseberry-mildew; an epidemic fungus disease now invading Europe". A second meeting was convened in the Imperial Institute, London, on 4 July 1907. Among the papers read was one by Mr A. D. Imms on "A disease of bees in the Isle of Wight", and one by Mr E. S. Salmon on "The American gooseberry-mildew, and the proposed legislative measures".

During 1908 two meetings were held, the annual general meeting at University College, London, on 15 April, and an Ordinary Meeting at the University of Edinburgh on 28, 29 and 30 July. A paper contributed to the London meeting by Mr C. Gordon Hewitt was entitled "The need of an organized enquiry into the feeding habits of British birds", and led to the moving of the following resolution: "That in the opinion of this Association it is desirable that a committee should be formed for the investigation of the feeding habits of British birds; the results of the work of such an Economic Ornithological Committee would be of very great importance in that it would obtain precise information concerning the economic habits of these birds." Such a committee later came into being and advanced in notable degree our knowledge of this problem. The Edinburgh meeting was rather sparsely attended but, among the fourteen papers contributed, were "Rats and their animal parasites", being the Presidential Address by Mr A. E. Shipley; "The action of Yohimbine on the generative organs", by Dr W. Cramer and Dr F. H. A. Marshall; and "Inbreeding and other experiments", by Prof. Cossar Ewart.

In 1909 an annual general meeting only was held, on 13, 14 and 15 July, in the School of Forestry of Oxford University. Among the papers read to the meeting were one by the President, Dr A. E. Shipley, dealing with grouse disease and entitled "The relation of certain Cestode and Nematode parasites to bacterial disease"; "The actual and possible application of recent discoveries in heredity to economic problems", by Mr A. D. Darbishire; "A successful curative treatment of Piroplasmosis", by Prof. G. H. F. Nuttall and Dr S. Hadwen; and "Investigations of the large larch sawfly,

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[1] The names of Original Members listed in the minute book number 26.

Nematus erichsoni", by Dr C. Gordon Hewitt. In the fourth Annual Report of the Council presented at this meeting it was noted that the total number of Members of all classes on 30 June 1909 was 132, and that the balance in the hands of the Hon. Treasurer amounted to £86. 19*s.* 1*d.*

During the years 1906–9, four annual parts of the *Proceedings of the Association* had been issued. With the appearance of the fourth part in July 1909, the *Proceedings* were discontinued, since arrangements had been made for Members of the Association to receive the *Journal of Economic Biology* which had been founded in 1905 by Mr W. E. Collinge.

In 1910 the ninth meeting of the Association was held on 6 and 7 July, in the University of Manchester. Among the papers read to the meeting were "On the place of economic zoology in a modern university", by Prof. S. J. Hickson, and "Wild bird protection", by Mr W. E. Collinge. At this meeting an Associate was elected for the last time. Associates remained on the membership list of the Association until 1914 but, in that year, they seem either to have become ordinary Members or to have discontinued their membership of the Association.

During 1911 meetings were convened at Birmingham University on 6 and 7 April, and in the rooms of the Linnean Society, London, on 7 July. Among the papers at the Birmingham meeting were "The training of economic entomologists", and "The standardization of economic nomenclature", both by Mr H. Maxwell Lefroy; and "The systematic recording of diseases of economic plants", by Dr J. H. Priestley. At the annual meeting in London official business only was transacted.

In 1912 the eleventh general meeting was held in the Royal College of Science, Dublin, on 28 and 29 March. Among the nine papers read were Prof. G. H. Carpenter's Presidential Address on "Biological training for agricultural students", and "Cereal breeding in Ireland", by Mr H. Hunter.

Until about 1912 the general organization and working arrangements of the Association, with secretarial headquarters in Birmingham and meetings held at irregular intervals in various educational centres, had worked fairly smoothly. About that time, however, the feeling became increasingly strong among the Officers and other active Members, that the Association would function more satisfactorily if its headquarters were in London and if its meetings were held at regular and more frequent intervals. Further, owing to difficulties which arose almost inevitably concerning the relationship between the Association and the *Journal of Economic Biology* which was owned privately by Mr Collinge, there was a widespread opinion that the Association should publish a scientific journal of its own.

After considerable discussion, both privately among Members and in Council, a Special Meeting was called in Birmingham on 13 September 1913, and the following resolutions were passed: "The meetings to be quarterly if material allows, and to be ordinarily in London, with one meeting in the Provinces annually. The management of the Association to be centred in London." "This meeting requests the Council to terminate the present arrangement with the Editor and Publisher of the *Journal of Economic Biology* regarding the supply of the *Journal* to Members with a view to the whole question of the relation of the *Journal* and the Association being reconsidered."

The twelfth general meeting was held on 30 December 1913, in the University of Liverpool and, in presenting their seventh Annual Report, the Council "have to report a number of proposed changes which they believe will lead to a renewal of

activity and the continued prosperity of the Association". The changes were in accordance with the resolutions noted above. Some thirty Members and visitors attended this meeting and ten papers were read.

These changes, which amounted to a major revolution in the conduct and policy of the Association, reflected the opinion general among the active workers in the society that the time was ripe for the Association to assume greater responsibility for the development of the science of applied biology and a more independent and prominent place in the organization of science. The Association had become the recognized society for all British workers interested in applied biology, but its provincial organization, and its dependence for the publication of its work on a private journal, were felt to be out of keeping not only with its accepted status but with the general development of the science in the country.

At this time one of the most active Members of the Association was Prof. H. Maxwell Lefroy, a man of unusual energy and vision, and it is largely to his credit that the revolution in the society was carried through successfully.

The transfer of the headquarters of the Association from Birmingham to London made it impossible for Mr W. E. Collinge to continue as Hon. Secretary. To mark their appreciation of his services the Members of the Association presented to Mr Collinge "a handsome piece of plate".

During the autumn and winter of 1913 negotiations were carried on with the Cambridge University Press with a view to the publication of an official journal of the Association. At the thirteenth general meeting of the Association held on 17 and 18 April 1914, in the Zoological Department of the Imperial College of Science, London, the President, Prof. Robert Newstead, announced the success of these negotiations and stated that Members would in future receive the *Annals of Applied Biology*, the "official organ of the Association". About thirty-five Members attended this meeting, forty new Members and two Honorary Members were elected, and eighteen papers were communicated. On the evening of 17 April, an Association Dinner took place in circumstances of considerable anxiety, since a few hours earlier Prof. Lefroy, who had been mainly responsible for its organization, had a serious motoring accident and was in a critical condition.

During the three or four preceding years the Association had made comparatively little progress, but this London meeting, the first to be held in the Imperial College of Science, marked the beginning of a period of renewed activity.

A second meeting was arranged in the Imperial College on 3 July 1914. Sixteen Ordinary Members and one Honorary Member were elected, and nine papers were read to the Association, including "Some aspects of deterioration in plants", by Dr E. J. Butler; "The improvement of Indian sugar canes", by Dr C. A. Barber; and "Insecticides; some considerations from a chemical standpoint", by Mr W. H. Nuthall.

In August 1914 the European war broke out. No further meetings of the Association were held during the year, but a Council meeting took place on Friday, 7 August 1914, and the following extract may be quoted from the Council minutes: "The next meeting was discussed: in view of the exceptional circumstances prevailing, the nation being at war with Germany and Austria as allies of France, Belgium, Russia, and Servia, it was decided to leave to the Secretary to settle, in September, if the proposed meeting be possible or not and for him to act accordingly." Although many Members were absent on active service and others were involved in various war-time activities,

it was decided to keep the Association in being and to maintain the *Annals of Applied Biology*.

A short meeting of the Association was convened on 20 December 1915, in the Imperial College, when eleven Members were present and official business only was transacted.

The next time the Association met was on 21 June 1917. This meeting took place in the Botanical Department of the Imperial College of Science, a Department which, thenceforward, became the unofficial but recognized home of the Association. Thirteen Members and four visitors attended and six communications were made.

In the minutes of a Council meeting held on 21 November 1917, the following appears: "It was proposed, possibly in conjunction with the Imperial Bureau, to make representations to the Minister of Reconstruction pointing out the great importance of Economic Biology and expressing the hope that this will not be lost sight of after the war." A minute of a Council meeting held on 20 March 1918 reads: "A draft of the Memorandum to be sent to the Ministry of Reconstruction was discussed. Dr Marshall said that it had been highly approved of both by Lord Harcourt and Sir Herbert Read."

In 1918 a meeting was called on 21 March; twelve Members and two visitors were present, and three papers were read.

During the war period Members engaged on war service were allowed to become "dormant Members", no subscriptions being required from them. This action and the difficulty of communicating with Members overseas greatly reduced the income of the Association, and the chief endeavour of the Council was directed to keeping the *Annals of Applied Biology* in being. Although the European war terminated in November 1918, conditions were very disturbed and many Members of the Association were not free to participate in its activities. A meeting was, however, held on 11 December 1918, when twenty Members and seven visitors were present, and four papers were communicated.

During the war period the Association had, with considerable difficulty, maintained itself in existence and had continued to publish the *Annals of Applied Biology*. By 1918, however, its activities and vitality had been reduced to a low ebb. The zoological side of the Association had always been the stronger and, in 1918, the botanical Members numbered less than one-third of the total membership. Some of the younger Members, particularly, felt that the Association could be revitalized by changing certain aspects of its conduct and general policy.

In January 1919, Mr W. B. Brierley submitted to the Council a memorandum containing proposals for the reorganization of the Association and the *Annals of Applied Biology*, and for a new orientation of general policy. Certain further proposals made by Prof. Lefroy were of interest in that they involved a change in the status of the Association from that of a scientific society to that of a body empowered by charter to grant some form of Degree or Diploma to those following the profession of applied biologist—on the need for which Prof. Lefroy held very strong views. After considerable discussion Mr Brierley's memorandum and Prof. Lefroy's proposals, together with the views of the Council thereon, were printed and circulated to all Members. The issues were discussed at ordinary meetings of the Association held on 20 March 1919 and 2 and 3 July 1919, and the proposals formulated in the memorandum were adopted.

A first desideratum was to increase the membership, especially on the botanical side, and, at the next meeting of the Association, on 10 and 11 December 1919, the names of sixty-nine botanists were proposed for membership. Five exhibits were shown and three papers were read. Furthermore, previous meetings of the Association had been confined to the reading of individual papers, but at this meeting was initiated the practice of holding symposia. This first symposium was on "The integration of mycological research with practice in agriculture, horticulture, and forestry", Sir Daniel Hall dealing with administration, Prof. V. H. Blackman with teaching, Dr E. J. Russell with agriculture, Mr F. J. Chittenden with horticulture, and Prof. William Somerville with forestry. The symposium was published in full as "Proceedings of the Association" in the *Annals of Applied Biology*.

In the early days of the Association it had been decided to form a library of books and reprints by exchange of the *Annals*, purchase, and donation. As the Association had no official buildings and only limited financial resources, the scheme did not progress. Further, with the founding of the Imperial Bureaux such a library was redundant. In 1920 the scheme lapsed and such literature as had accumulated was deposited with the Imperial Bureau of Entomology, certain duplicates being sent to the Rothamsted Experimental Station.

After 1920 the Association showed slow but steady progress. Six to ten meetings per year were convened during the winter months in London, with an occasional meeting in the Provinces; a field meeting was, with rare omission, held each summer in some research station, botanic garden, or other place of applied biological interest; and frequently, also, a half-day excursion was arranged each autumn to some centre of research in applied biology.

During 1920 three London meetings were held, and visits were paid to the Royal Botanic Gardens, Kew, and to the Rothamsted Experimental Station. Twelve papers were communicated to the Association, and a discussion took place on "The reclamation of waste land". The average attendance at meetings was about seventy-three.

The year 1921 commenced a period of increased activity of the Association. Eight London meetings were arranged, and a day was spent at Reading in the trial grounds of Messrs Sutton and Sons, and in the Agricultural Botanic Garden of Reading University. Ten papers were read before the Association; the retiring President, Sir David Prain, delivered his Presidential Address on "Some relationships of economic biology", and general discussions or symposia were held on the following subjects: "The physiology of the infection process", "Meteorological conditions and disease in plants", "The resistance of the normal and injured plant surface to the entry of pathogenic organisms", and "The importance of scientific research in forestry and its position in the Empire". The average attendance at meetings was about seventy-three.

The period 1919–21 during which the Association was reconstructing itself were years of anxiety and difficulty. Perhaps only the Officers and Council during that period realize fully the debt the Association owes to Sir David Prain, President of the Association from 1919 to 1921. A minute from the Council meeting held on 9 December 1921 may be quoted: "Sir David Prain intimated his desire to surrender the Office of President of the Association. The Council expressed their grateful appreciation of the wise guidance with which the retiring President had conducted the Association through the very difficult years of his term of Office." An equally good friend and wise guide during a further difficult period was Sir E. B. Poulton, President for the years 1922–3.

In 1922 six London meetings were arranged, and a meeting extending over two days was held in Manchester at the Victoria University and the Shirley Cotton Research Institute. Seven papers were read before the Association and general discussions or symposia took place on the following subjects: "Virus diseases in plants and animals", "Sea fisheries research", and "Genetics in relation to applied biology". The average attendance at meetings was about sixty-three.

In 1923, seven London meetings were arranged, and a day was spent at Cambridge visiting the School of Agriculture, Plant Breeding Institute, University Farm, and National Institute of Agricultural Botany. Nine papers were read to the Association and general discussions or symposia were held on the following subjects: "Partial sterilization of soil", "Pathogenic Protozoa in plants and animals", and "The nature of ultramicroscopic viruses". The attendance at meetings averaged about fifty-nine.

Commencing with the year 1924 the Annual Reports of the Council of the Association and the Hon. Treasurer's Statement of Accounts have been published in full in the *Annals of Applied Biology*, part 2 of each volume. The following data have been extracted from these Annual Reports:

(a) *Presidential Addresses*

1924—Prof. E. B. Poulton, "The relation of pure and applied biology"; 1926—Prof. V. H. Blackman, "Recent work in plant physiology and its relation to applied biology"; 1928—Mr J. C. F. Fryer, "Legislation in England against diseases and pests of plants"; 1930—Dr E. J. Butler, "Some aspects of the morbid anatomy of plants"; 1932—Dr A. D. Imms, "Temperature and humidity in relation to insect control"; 1934—Prof. W. B. Brierley, "Some viewpoints of an applied biologist"; 1936—Dr T. Goodey, "Some applied biological aspects relating to plant parasitic nematodes"; 1938—Dr J. Henderson Smith, "Some recent development in virus research".

(b) *General discussions and symposia*

1924—"Vegetative propagation"; "Cold storage problems"; "Genetics and the stock-breeder"; General principles that should underlie government action regarding fungicides and insecticides: 1925—"Research on wart disease of potatoes"; "The use of sulphur as a fungicide"; "The place of the systematist in applied biological work": 1926—"Forestry in relation to science"; "Crown-gall of plants": 1927—"Plant alkaloids"; "Research in applied biology at the South Eastern Agricultural College, Wye"; "Agricultural problems in tropical Africa"; "Foot and mouth disease of cattle": 1928—"Work of the Plymouth Marine Laboratory"; "Work of the Forest Products Research Laboratory, Princes Risborough"; "Relation of environmental conditions to disease in plants": 1929—"Work of the Empire Marketing Board"; "Agricultural science in Australia"; "Work of the National Institute for Research in Dairying, Reading"; "Research on infestation of stored products"; "The incidence and control of apple scab": 1930—"Biological control of injurious insects and weeds"; "Nutrition of fruit trees"; "Factors influencing yield of cereal crops"; "Purification of waste waters from sugar beet factories": 1931—"Biological races and their significance in evolution"; "Economic applications of microbiology"; "Training of biologists for economic posts"; "Laboratory tests of fungicides": 1932—"Decay of stonework"; "Fungal deterioration of stored products and dairy produce"; "Decomposition of plant materials": 1933—"Pests of mushrooms"; "Physiological

disorders of glasshouse crops and fruit trees": 1934—"Plant pathological problems in the tropics": 1935—"Nitrification processes in the soil"; "Sawfly problems"; "Glasshouse problems at the Experimental and Research Station, Cheshunt"; "Insect population studies": 1936—"Problems raised by the woolly aphis of the apple"; "Effect of various conditions on the resistance of apples to fungal attack": 1937—"Recent work on the death-watch beetle, *Xestobium rufovillosum*"; "Recent developments in fumigation"; "The wireworm problem": 1938—"Chemical weedkillers"; "Fresh-water biology and its applications"; "Apple canker".

(c) *Provincial and field meetings and visits*

1924—Leeds University; Wembley Exhibition, London: 1925—Edinburgh University; nurseries of Messrs Bunyard, Maidstone: 1926—Experimental and Research Station, Cheshunt: 1927—South-Eastern Agricultural College, Wye; Imperial Institute, London: 1928—Long Ashton Research Station, Bristol; Royal Botanic Gardens, Kew: 1929—London Docks and Warehouses: 1931—Parasite Station of the Imperial Institute of Entomology, Farnham Royal: 1932—Biological Field Station of the Imperial College of Science, Slough: 1933—Reading University: 1934—Wellcome Physiological Research Laboratories, Beckenham; London School of Hygiene and Tropical Medicine: 1935—East Malling Research Station; Lister Institute of Preventive Medicine, London: 1936—Royal Horticultural Society's Gardens, Wisley; Wellcome Museum of Medical Science, London: 1937—Experimental and Research Station, Cheshunt; Gardens of the Zoological Society of London: 1938—Forest Products Research Laboratory, Princes Risborough; Gaumont British Film Studios, London.

From 1917 until 1935 all ordinary meetings of the Association in London were convened in the botanical lecture theatre of the Imperial College of Science. Council meetings are still held in the Botany Department but, since 15 November 1935, nearly all ordinary meetings of the Association have taken place in the metallurgy lecture theatre of the Imperial College. Until 1931 the London meetings were held on Friday afternoons but, in that year, the present practice was adopted of holding two full-day meetings and two half-day meetings per year. During 1924 Members first met for tea and social intercourse after the meetings.

From its inauguration in 1904 the Society had been known as the *Association of Economic Biologists*. After considerable discussion and the taking of a referendum of all Members, it was decided at an extraordinary general meeting held on 24 February 1934, to change the original title to the *Association of Applied Biologists*.

II. The *Annals of Applied Biology*

When the Association of Economic Biologists was inaugurated in November 1904 the publication of an official journal was not envisaged. It was decided to issue annual *Proceedings of the Association* containing the Annual Reports of the Council and the Hon. Treasurer, notices of the meetings, and abstracts of the papers communicated to the society. Four such *Proceedings* were published for the years 1905–8.

In 1905 Mr W. E. Collinge, Hon. Secretary of the Association, founded the *Journal of Economic Biology*, part 1 of vol. I appearing under date of 15 November 1905. The following extracts may be quoted from an Editorial: "It has for some time been apparent that workers in Economic Biology have found difficulty in obtaining

publication of their papers, and particularly so if good illustrations were required. With the foundation of an Association of Economic Biologists in the United Kingdom, such papers will naturally increase in number, and whilst this body is able to deal with the publication of the smaller papers larger ones requiring carefully executed plain or coloured lithographic plates are still unprovided for. It is intended in this journal to offer a medium for such work."

The *Journal of Economic Biology* belonged to Mr W. E. Collinge and not to the Association, and the Association, as such, had no financial or editorial interest in the publication. The *Journal* was, however, quite generally regarded as the unofficial organ of the Association. It was edited by Mr W. E. Collinge, Hon. Secretary of the Association, with the co-operation of Prof. A. H. R. Buller, Prof. G. H. Carpenter, Mr Robert Newstead, and Mr A. E. Shipley, one a Vice-President of the Association, and all active Council Members of the Association. Furthermore, many of the papers read to the Association, including some of the Presidential Addresses, were published in the *Journal of Economic Biology.*

In 1908 the Council of the Association entered into an agreement with Mr Collinge whereby he agreed to supply the *Journal* to Members of the Association and "to publish the various papers read before the Association, if the same should be deemed worthy of publication". The financial and general control of the *Journal* remained, as hitherto, in the hands of its proprietor, Mr W. E. Collinge. The only changes in the *Journal* were that Prof. Percy Groom, newly elected to the Council of the Association, was added to the Editorial Board and that the *Proceedings of the Association*, hitherto published annually as separate pamphlets were, from 1909 to 1912, published in the *Journal.*

The annual subscription to the Association was 10s. 6d. but, in accordance with the new arrangement whereby Members received the *Journal of Economic Biology* free, the subscription was raised to 21s. The arrangement came into force as from January 1910, with vol. v of the *Journal,* and continued until 1913.

This arrangement, although convenient at the time, did not prove satisfactory. It became obvious that it was not to the best interests of the Association that its *Proceedings* and scientific papers should be published in a journal unofficially sponsored by the Association but over which the Association possessed no editorial or financial control. At a special general meeting of the Association held on 12 September 1913, it was decided that the arrangement with Mr Collinge should lapse with part 4, vol. VIII, December 1913, of the *Journal,* and that the Association should manage its own official publication.

The *Journal of Economic Biology* appeared during 1914 under the Editorship solely of Mr W. E. Collinge, and, at the end of 1915, was discontinued. In 1916 it appeared as the *Journal of Zoological Research,* edited by Mr W. E. Collinge, but finally disappeared with no. 4, vol. III, December 1918.

The last *Proceedings of the Association* to be published in the *Journal of Economic Biology* appeared in part 4, vol. VII, December 1912.

The decision of the Association to publish a scientific journal of its own required faith and courage, since the financial position of the Association was far from strong, and the membership had actually dwindled from 132 in 1909 to 105 in 1913. The Council's Annual Report presented to the twelfth general meeting on 30 December 1913, records that "The total receipts up to December 1913 amounted to £66. 12s. 0d.

whilst the total expenditure for the same period amounted to £88. 13s. 3d., leaving a balance in the hands of the Hon. Treasurer of £100. 7s. 1d. There is also an outstanding balance of £43. 13s. 0d. for subscriptions owing". Nevertheless, during 1913, negotiations were entered into with the Cambridge University Press and, in May 1914, part 1, vol. I of the *Annals of Applied Biology* appeared.

Not only the Association but the science of applied biology are deeply in debt to Prof. Maxwell Lefroy whose energy and vision were largely responsible for the successful inauguration of the *Annals*.

The *Annals of Applied Biology* was the property of the Association, a scientific journal over which the Association possessed full editorial and financial control. The Hon. Secretary of the Association, Prof. H. Maxwell Lefroy, was appointed Hon. Editor, and in conducting the new *Annals* he was assisted by an Editorial Committee consisting of Prof. B. T. P. Barker, Dr S. E. Chandler, Mr F. J. Chittenden, Prof. F. W. Gamble, Prof. Percy Groom, Dr A. D. Imms, Prof. Robert Newstead, and Prof. J. H. Priestley.

In an Editorial to the first number of the *Annals* Prof. Maxwell Lefroy indicated the general policy to be pursued, and extracts from this may be quoted: "The Association of Economic Biologists was founded ten years ago and commences herewith the publication of a journal devoted to the special interests of its Members. During this period its scope has broadened and the *Annals of Applied Biology* is intended to cover the ground in Applied Biology which is not now covered by special journals such as those dealing with agricultural science, parasitology, genetics, and medical science." "All papers which bear on the scientific problems of applied biology will be welcome; we have no place for purely systematic work which is amply provided for elsewhere, nor for faunistic work as such." "We hope to attract not only the more solid scientific contributions but also notes of progress, of interesting achievements, of practical problems, as they present themselves to Members in the various parts of the Empire."

From 1914 to 1924 (vols. I–XI) the *Annals* was published somewhat irregularly as material became available. Two parts of each volume appeared as separate issues, and the two remaining parts as a double number.

With the completion of vol. II of the *Annals* issued in April 1916, Prof. H. Maxwell Lefroy relinquished the Hon. Editorship and Mr E. E. Green, lately Government Entomologist in Ceylon, was invited by the Council to undertake the work. There was no change in editorial policy or in the Editorial Board but, in December 1919, the double number containing parts 2 and 3 of vol. VI appeared under the Hon. Editorship of Mr E. E. Green "with the assistance of the Council".

With the completion of part 4, vol. VIII, February 1921, Mr E. E. Green relinquished the Hon. Editorship and the Council of the Association decided to effect certain rearrangements in the conduct and policy of the *Annals*. Mr W. B. Brierley was invited to become Hon. General and Botanical Editor, and Mr D. Ward Cutler Hon. Zoological Editor. A "Publications Committee" was appointed consisting of the Hon. Treasurer, two Council Members, and two Ordinary Members (A. D. Imms, V. H. Blackman, E. E. Green, A. W. Hill, R. T. Leiper).

Since 1921 there has been no change in the Hon. General and Botanical Editorship of the *Annals*. Mr D. Ward Cutler remained Hon. Zoological Editor until 1932, when he was succeeded by Prof. J. W. Munro in 1933, and by Mr C. T. Gimingham in 1934.

Vol. I of the *Annals* was published in May and July 1914, and January 1915, i.e. it contained investigations completed prior to the outbreak of the war in August 1914. The volume comprised 406 pages and 27 plates. The early months of the war left a sufficient number of workers to carry on, and in the minutes of a Council meeting held on 27 January 1915, the following appears: "The question of the future of the *Annals of Applied Biology* was discussed. It was decided to issue four numbers of reduced size." Parts 1, 2 and 3 of vol. II were issued in May and July 1915, but in the minutes of a Council meeting held on 29 October 1915, the following appears: "It was decided to suspend publication of *Annals*, II, 4, till the General Meeting had expressed an opinion, unless it was found that there were funds sufficient on this year's (1915) subscriptions and sales to admit of its immediate issue." Fortunately, the Royal Society of London generously made a grant of £50 towards the publication of the *Annals*. Part 4 was, therefore, issued in April 1916, the total volume containing 292 pages and 39 plates.

Difficulties, however, continued to increase. Vol. III of the *Annals,* published June 1916, and January and April 1917, was reduced to 204 pages and 24 plates but, even so, it cost more than the depleted funds of the Association could stand. Again, the Royal Society of London generously granted a sum of £50 towards publication expenses, and vol. IV, comprising 239 pages and 12 plates, was issued September and December 1917, and March 1918. The Association is greatly indebted to Prof. Newstead, then President, since it was in no small measure due to his exertions that the claims of the Association were brought successfully to the notice of the Royal Society.

With the *Annals* to carry on, finance was the chief preoccupation of the Council during these years, but in the minutes of a Council meeting held on 21 November 1917, the following appears: "It was further proposed by Dr Marshall and seconded by Dr MacDougall that in view of the anticipated balance at the end of the year, no application for a further grant to the Royal Society should be made. This was also approved." A minute of the Council meeting held on 16 October 1918, reads: "The Treasurer made a statement as to the satisfactory financial position of the Association. ...After some discussion it was not considered advisable to invest the £100 now held in reserve or to raise the price of the *Annals*." Vol. V of the *Annals*, comprising 281 pages and 11 plates, was issued July and October 1918, and April 1919.

With the release of men from the army or war services after November 1918, the demand upon the *Annals'* space began to increase. Further, the Association for the first time in many years possessed a little money in reserve. Accordingly, vol. VI of the *Annals*, published September and December 1919, and April 1920, was enlarged to 356 pages and 10 plates. The increased costs of production of this volume caused disquiet in the mind of the Hon. Treasurer. At a Council meeting held on 5 November 1919, he presented a statement "respecting the financial position of the *Annals*", suggested certain economies, and "Recommended that an attempt should be made to obtain a grant in aid from the Royal Society or from the Development Commission of the Board of Agriculture". It was decided to apply for a sum of £200. At a general meeting of the Association held on 4 June 1920, the "Chairman announced that the Development Commissioners had recommended a grant to the Association up to £200 and that this recommendation had been sent to the Treasury".

This generous grant practically saved the *Annals* and the Association is all the more grateful for its receipt since the Development Commissioner's policy at that time

was contrary to making grants for publication. The Association is deeply indebted to Sir David Prain, then President, and Mr J. C. F. Fryer, then Hon. Treasurer, for their successful efforts in obtaining this grant.

The money was received, and vol. VII of the *Annals* issued September and December 1920, and February 1921, was enlarged to 431 pages and 26 plates, three of the latter being in lithograph and one in colour. This was by far the largest and most opulent volume of the *Annals* the Association had yet produced and the expenses of publication were correspondingly large, especially as general costs of production were rising steeply during this period.

Parts 2 and 3, the double number of vol. VII, which contained 204 pages and the four special plates, alarmed the Hon. Treasurer and, at a Council meeting on 10 December 1920, "The question of the publication of the *Annals of Applied Biology* was discussed at some length and a sub-committee was appointed to consider the matter and report to the next Council meeting". Their report was presented at a Council meeting on 28 January 1921. Among their recommendations were the raising of the annual subscription to the Association from 21*s.* to 25*s.* and of the subscription price of the *Annals* from 33*s.* 6*d.* to £2, a reduction in author's free reprints from 50 to 25, and "That for the present no illustrations in the *Annals of Applied Biology* other than text-figures be paid for by the Association save under exceptional circumstances". "Material financial retrenchment" was imperative if the Association were to remain solvent, and it was decided to raise a "Publications Fund for the purpose of bridging over the difficulty of carrying on the *Annals of Applied Biology*".

Vol. VII was the last produced under the Hon. Editorship of Mr E. E. Green and the new Hon. Editors, Mr W. B. Brierley and Mr D. Ward Cutler, elected in December 1920, were faced with the task of producing the *Annals* in the Association's greatly reduced circumstances. By dint of stringent economy and drastic cutting, and the receipt of "Grants in aid of publication" from eight of the seventeen authors contributing manuscripts, vol. VIII was produced and was issued June, August, and November 1921. This volume, which contained only 219 pages and 4 plates, was the nadir of the *Annals of Applied Biology*. In an "Editorial Note" to part 1 of vol. VIII of the *Annals* the new Hon. Editors outlined certain of the economies considered necessary but stated, "It is hoped that the reductions outlined above will only be operative for the current year since it is anticipated that in 1922 it will be possible to revert to improved conditions".

During 1922 financial conditions, although still very difficult, were slightly better than in the previous period, but this improvement was offset by the fact that, with the return of the country to normal, more scientific work was carried out and more manuscripts were submitted for publication in the *Annals*. The "Publications Fund" had reached some £70 and, by the exercise of strict economy, and ruthless editorial demands for "Grants in aid of publication", vol. IX of the *Annals* was produced. Twenty-four papers were published and the *Annals*, issued April, June, and November 1922, contained 359 pages and 14 plates.

The Association had survived the crisis and maintained its *Annals*, and, although passing years saw slight changes of fortune, the progress of the Association and its *Annals* has been well maintained. Vol. X, published February, July, and December 1923, was increased to 454 pages and 22 plates, and vol. XI, issued April, July, and October 1924, still further enlarged to 519 pages and 16 plates. In this volume was

initiated the practice of publishing the Hon. Treasurer's "Statement of Accounts" for the preceding year. The Statement for the year ending 31 December 1923 showed a cash balance of £101. 18s. 5d. and the sum of £400 invested in Savings Certificates, which has remained as the nucleus of a reserve fund.

So far two separate numbers and one double number of the *Annals* had been published somewhat irregularly each year. During 1924 the Hon. Editors made arrangements with the Cambridge University Press for the issue of four separate parts per year and, in 1925, the parts of vol. XII appeared in February, May, July, and November. The volume, the largest yet published by the Association, contained 549 pages and 18 plates.

Commencing with 1926 the four parts of the *Annals* have, with one or two exceptions only, and then merely by a matter of a few days, appeared regularly in the months of February, May, August, and November. Furthermore, unless papers were unusually long or required an unusual number of plates, no request has been made for "Grants in aid of publication", and no subsidy has been necessary from outside sources. Vol. XIII (1926) comprised 645 pages and 20 plates. Vols. XIV–XXI (1927–34) had an average size of 690 pages and 36 plates. Vol. XVII, containing 810 pages and 50 plates, was rather a special effort in view of the holding of the Fifth International Conference of Botany at Cambridge in 1930.

Commencing about the year 1933 the number of manuscripts submitted to the Hon. Editors began to increase and this resulted in delay in the publication of research. Vol. XXII (1935) was, therefore, increased to 820 pages and 35 plates. Part 2 of this volume contained a paper of 82 pages by H. Martin on "The standardization of petroleum and tar oils and preparations as insecticides" which the Council reprinted for sale as a separate issue. Vol. XXIII (1936) was increased to 921 pages and 39 plates; and Vol. XXIV (1937) to 940 pages and 51 plates. Vol. XXIV of the *Annals* was the largest ever published by the Association and contained 59 scientific papers, a "Proceedings of the Association" of 15 pages, reviews of 44 books, and the Reports of the Council and Hon. Treasurer.

As the official scientific journal of the Association of Applied Biologists the *Annals of Applied Biology* is devoted to the special interests of Members of the Association. The great majority of the papers published in the *Annals* since its commencement in 1914 have been contributed by Members or by scientific workers who later became Members. Not infrequently, however, papers by non-members and by workers in various countries outside the British Empire have been accepted.

In the first 25 volumes of the *Annals of Applied Biology* (1914–38) the total number of papers, including "Proceedings", notes, and longer obituary notices, is 960. These may roughly be classified as follows:

General applied botany (including non-parasitic diseases)	131
Mycology and fungal diseases	160
Bacterial diseases	50
Viruses and virus diseases	108
General applied zoology	26
Entomology and insect pests	250
Plant protection (fungicides, insecticides, etc.)	99
Microbiology (of food, soil, etc.)	62
Helminthology and nematode diseases	20
Apparatus (botanical and zoological)	10
General (botany, zoology, obituary notices, etc.)	44

Earlier volumes of the *Annals* contained occasional book reviews, but in 1924 the Hon. Editors, in response to suggestions from workers in overseas parts of the Empire, enlarged this service of the *Annals*. Commencing with Vol. XI the average number of book reviews per volume has been 18.

Free copies of the *Annals of Applied Biology* are supplied as follows:

Copyright Libraries. 6 copies.

Syndics, Cambridge University Press. 1 copy.

Botany Department, Imperial College of Science and Technology, London; in grateful recognition of its hospitality. 1 copy.

During the whole period of its existence the *Annals of Applied Biology* has been produced and published for the Association by the Cambridge University Press. No praise can be too high for the technical efficiency or artistry of its production, or for the consummate skill shown by the Press readers.

III. CONCLUSION

The development of the Association shows certain stages which may be related to more general developments in the country.

Prior to 1904 a mere handful of workers in Applied Biology was scattered in various appointments throughout the United Kingdom. There was little opportunity or encouragement for research in this field, since few facilities existed, and the emphasis in Universities and other educational institutions was laid primarily on pure science. The only agricultural research institution in the country was the Rothamsted Experimental Station where biology had not gained a foothold.

The Association was founded in November 1904. The period 1905 to about 1909 was one of rapid growth of the Association, and its practice of holding meetings in various educational centres throughout the United Kingdom—Birmingham, Liverpool, Cambridge, London, Edinburgh, Oxford, Manchester, Dublin—must have had great propaganda value for the subject of applied biology, and, in large part, may be held responsible for the steadily increasing recognition of the importance of the subject.

This recognition received practical outcome with the passing of the Development Fund Act in 1909, whereby a sum of two million pounds was set aside for the development of agricultural education, and particularly of agricultural research. During the next five years the scheme of agricultural research institutes, in principle almost as it exists to-day, came into being. The scheme had, however, barely come into active operation when the war broke out in August 1914.

Until about 1909 the Association had been finding itself, energizing and proselytizing in all directions. The period 1909 to about 1913 was also one of considerable activity in the Association, but this activity was directed inwardly rather than to any specific external new developments. The Association reorganized itself, settled down in London, and decided to produce the *Annals of Applied Biology*.

With 1914 the schemes of agricultural and horticultural research in the country began to function adequately and there was a great spirit of optimism throughout the whole field of applied biology. This was reflected in the increased membership and activity of the Association, which seemed about that time to assume a new lease of life, and in the founding of the *Annals of Applied Biology*. In August, came the European war and this promise came to an abrupt end.

During the war years the Association maintained itself in existence but it became a society with one preoccupation—the production of the *Annals*. It was not until after 1919 that conditions had returned sufficiently to normal to enable the Members to come together and, in the meantime, their sole link was the *Annals*.

The period 1919 to 1922–3 was one of slow recovery and general reorganization in the agricultural research schemes of the country. With the repeal of the Corn Production Acts in 1921 a sum of £850,000, to be spread over five years, was appropriated for agricultural education and research. This permitted the adequate development of schemes which had fallen into abeyance and the inauguration of new schemes and, about 1922–3, the effects began to be seen in an increased number and activity of research workers and an expanding volume of research. During this period the Imperial Bureau of Entomology, founded some years earlier, resumed and increased its activity, the Imperial Bureau of Mycology came into being, an Institute of Plant Pathology was founded at the Rothamsted Experimental Station, the Ministry of Agriculture's Plant Pathological Laboratory was established at Harpenden, and other important developments occurred.

In the Association the period 1919 to 1922–3 was one of great difficulty. In the winter of 1918–19 the Association had reached its lowest ebb and the *Annals* was almost defunct, but, during 1919–21, the Association was reorganized. It survived the crisis and by 1922–3 was in a condition to meet the accelerating demands for regular scientific meetings and for increased facilities for publication.

In the country the next six years (1923–8) was a period of expansion. The agricultural and horticultural research institutes developed rapidly, and great encouragement was received from the Imperial Agricultural Conference of 1926, and the establishment of that fairy Godmother, the Empire Marketing Board, which initiated and developed numerous research schemes, including that of virus diseases of plants. Additional Imperial Bureaux were established and began to make their influence felt. The Department of Scientific and Industrial Research expanded rapidly.

In the Association the period 1923–8 was one of great activity; numerous and successful meetings were held, and the *Annals of Applied Biology* from a volume of 359 pages and 14 plates in 1922 expanded at almost a steady rate per year to a volume of 707 pages and 39 plates in 1928. To take virus research as one example. In the fourteen volumes of the *Annals* published from 1914 to 1927 there were only nine papers on viruses and virus diseases. About 1927 the virus research schemes came into full working order, and, in the eleven volumes of the *Annals* published from 1928 to 1938, the papers on this subject number about one hundred.

Since 1928 the Association and its *Annals* have experienced fluctuations of fortune but the general trend of membership, activity at meetings, and publication has been in an upward direction. There have been no marked changes in the constitution of the Association or, save in the steady widening of its interests, new and noteworthy developments in its general activities. Perhaps the most striking development has been the increase in size and scientific value of the *Annals of Applied Biology*.

The following Members have served as Officers of the Association of Applied Biologists in the capacities and for the periods noted:

I. *Presidents*

Mr Fred V. Theobald	1904–1906	Mr J. C. F. Fryer	1926–1927
Sir A. E. Shipley	1907–1909	Dr E. J. Butler	1928–1929
Prof. G. H. Carpenter	1910–1913	Dr A. D. Imms	1930–1931
Prof. Robert Newstead	1914–1917	Prof. W. B. Brierley	1932–1933
Sir J. B. Farmer	1918	Dr T. Goodey	1934–1935
Sir D. Prain	1919–1921	Dr J. Hendersom Smith	1936–1937
Sir E. B. Poulton	1922–1923	Mr C. T. Gimingham	1938–
Prof. V. H. Blackman	1924–1925		

II. *Vice-Presidents*

Sir P. Manson	1905–1915	Dr E. J. Butler	1924–1925
Sir A. E. Shipley 1905–1907,	1914–1919	Dr A. D. Imms	1924–1927
Prof. W. Somerville 1905–1909,	1920	Dr G. H. Pethybridge	1926–1927
Mr Fred V. Theobald	1907–1910	Dr J. Waterston	1928–1930
Sir E. B. Poulton	1909–1913	Prof. F. T. Brooks	1928–1929
Sir J. B. Farmer	1910–1913	Prof. W. B. Brierley	1930–1931
Prof. G. H. F. Nuttall	1910–1913	Dr W. R. Thompson	1930–1932
Sir Ronald Ross	1910–1911	Mr A. D. Cotton	1932–1934
Prof. S. J. Hickson	1911–1920	Prof. W. Brown	1933, 1935
Prof. G. H. Carpenter	1914–1920	Prof. J. W. Munro	1933
Prof. R. Stuart MacDougall	1914–1919	Dr C. B. Williams	1934–1935
Prof. G. Stanley Gardiner	1917–1920	Dr R. C. Fisher	1936
Mr A. G. L. Rogers	1920–1921	Dr S. P. Wiltshire	1936
Sir G. A. K. Marshall	1920–1922	Mr C. T. Gimingham	1937
Prof. V. H. Blackman	1922–1923	Dr H. Martin	1938
Sir E. J. Russell	1923	Dr H. Wormald	1938

III. *Hon. Treasurers*

Mr Herbert Stone	1904–1909	Dr A. D. Imms	1920–1930
Prof. R. T. Leiper	1910–1913	Dr J. Henderson Smith	1931–
Mr J. C. F. Fryer	1914–1919		

IV. *Hon. Editors*

Prof. H. Maxwell Lefroy	1914–1915	Prof. J. W. Munro	1933
Mr E. E. Green	1916–1920	(Zoological)	
Prof. W. B. Brierley	1921–	Mr C. T. Gimingham	1934–
(General and Botanical)		(Zoological)	
Mr D. Ward Cutler	1921–1932		
(Zoological)			

V. *Hon. Secretaries*

Mr W. E. Collinge	1904–1913	Prof. S. G. Paine	1923–1926
Mr W. G. Freeman	1910	Dr T. F. Chipp	1927
Dr S. E. Chandler	1911–1913	Prof. J. W. Munro	1928–1932
Prof. H. Maxwell Lefroy	1914	Prof. W. Brown	1928–1932
Prof. P. Groom	1915–1916	Prof. R. H. Stoughton	1933–1935
Dr S. A. Neave	1917–1921	Mr G. Fox Wilson	1933–
Prof. W. B. Brierley	1919–1922	Mr W. P. K. Findlay	1936–
Dr J. Waterston	1922		

Prof. F. V. Theobald.   President 1904–1906

Photo. Elliott & Fry

Sir Arthur E. Shipley.   President 1907–1909
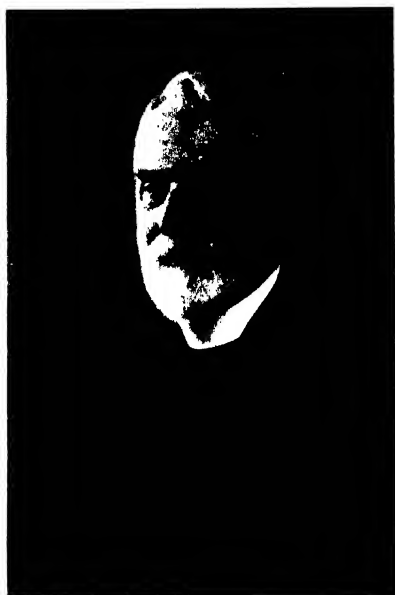
Photo. Gardiner

Prof. G. H. Carpenter.   President 1910–1913
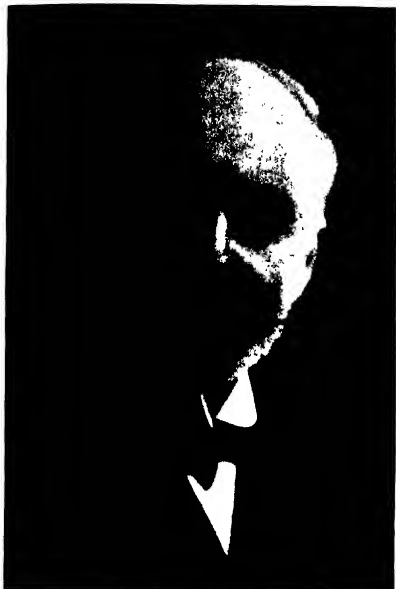
Prof. Robert Newstead. President 1914-1917
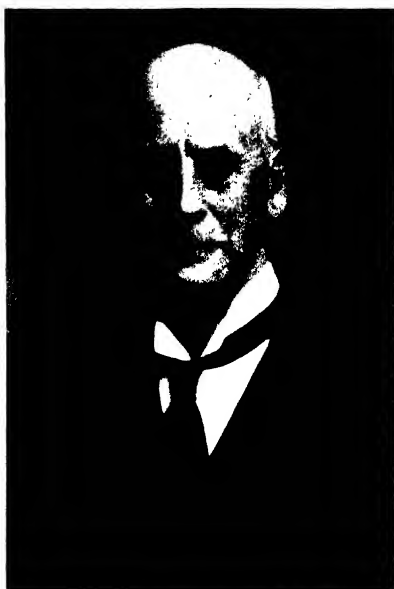
Sir John B. Farmer. President 1918

Sir David Prain. President 1919-1921

Sir Edward B. Poulton. President 1922-1923
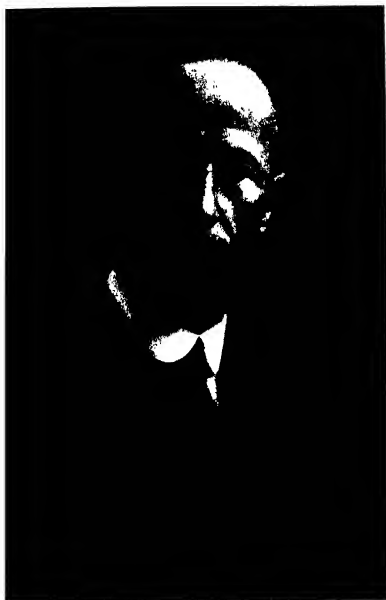
Photo. Elliott & Fry

Prof. V. H. Blackman. President 1924–1925
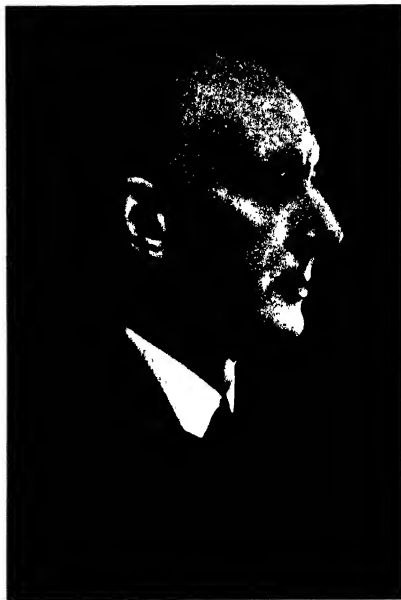
Mr J. C. F. Fryer. President 1926–1927

Photo. Elliott & Fry

Dr E. J. Butler. President 1928–1929

Photo. Lafayette

Dr A. D. Imms. President 1930–1931

Photo. Corbett

Prof. W. B. Brierley. President 1932–1933
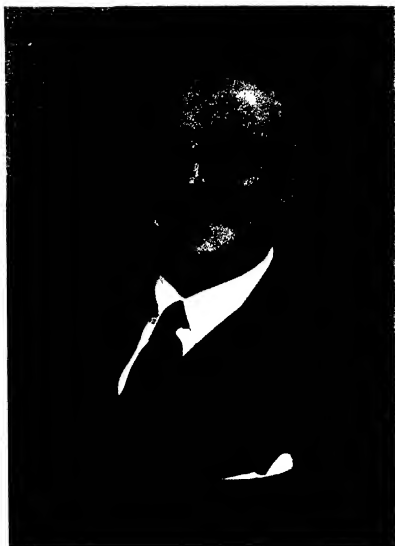
Dr T. Goodey. President 1934–193
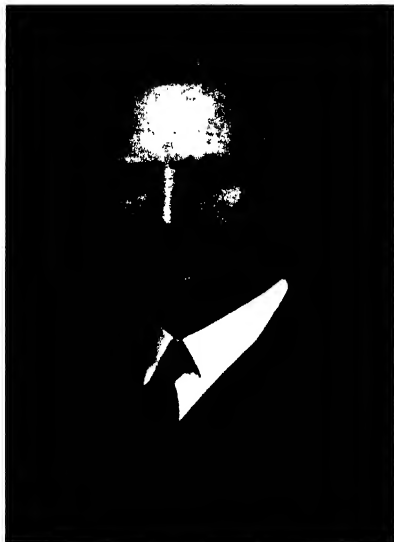
Photo. Elliott & Fry

Dr J. Henderson Smith. President 1936–1937

Mr C. T. Gimingham. President 1938–

BRIERLEY.—The Association of Applied Biologists and the Annals of Applied Biology—
A Retrospect (1904–38) (pp. 178–195)

Honorary Members of the Association

A. Past Honorary Members:

	Date of election		Date of election
Prof. R. Blanchard	1905	Prof. A. Berlese	1905
Dr A. Looss	1905	Prof. G. Cuboni	1914
Prof. G. L. Neumann	1905	Prof. A. Railliet	1914
Prof. J. Ritzema-Bos	1905	Prof. W. Johannsen	1921
Prof. N. A. Cholodkovsky	1905	Prof. M. W. Beijerinck	1925

B. Honorary Members, 1938:

Dr L. O. Howard	1905	Prof. Chevalier	1928
Dr A. D. Hopkins	1905	Prof. P. Silvestri	1928
Prof. P. Marchal	1914	Prof. N. I. Vavilov	1929
Prof. L. R. Jones	1923	Prof. E. Gäumann	1934
Prof. N. H. Nilsson-Ehle	1923	Dr B. P. Uvarov	1934
Prof. O. Appel	1924	Prof. K. Escherich	1934

Membership of the Association

Year	Number	Year	Number
1905	68	1923	255
1906	80	1924	257
1907	97	1925	243
1908	129	1926	241
1909	132	1927	255
1910	118	1928	283
1911	104	1929	289
1912	104	1930	301
1913	105	1931	303
1914	?	(Members in arrears removed)	
1915	?	1932	252
1916	?	1933	285
1917	?	1934	291
1918	?	1935	308
1919	130	1936	310
1920	208	1937	314
1921	247	1938	326
1922	246		

NOTES ON THE PLATES

With the exception of Prof. G. H. Carpenter the portraits are approximately contemporaneous with the Presidential terms of Office.

No early photograph of Prof. Carpenter was available and his portrait is reproduced from a photograph taken in 1938.

I am greatly indebted to Elliott & Fry, Ltd., Lafayette, Ltd., and J. Russell & Sons, for permission to reproduce portraits taken by them.

I also wish to express my gratitude to those who have helped me to complete the Presidential portrait gallery.

REVIEWS

Handbuch der Pflanzenkrankheiten. Herausgegeben von Prof. Dr O. APPEL. Bd. VI. *Verhütung und Bekämpfung der Pflanzenkrankheiten.* 2 Lief. Pp. 289–576. Berlin: Paul Parey. 1938. 16.60 Rm.

Dr Appel has found it necessary to issue the comprehensive textbook on Plant Protection which he has added, as vol. VI, to the original Sorauer, in parts, of which this is the second. The first instalment was reviewed in the *Annals*, 1937, **24**, 675. The volume will be completed in four to five such parts which cannot be purchased singly. Consequently the second part opens in the middle of a discussion by Dr W. Trappmann on the use of mechanical barriers for crop protection. He continues to describe, in 44 pages, other physical methods whereby damage by pest or weather may be prevented or by which pests may be trapped. The subject is treated so completely and thoroughly that it may be deduced that this chapter was written towards the close of 1935 but it is a pity that the opportunity of including some references up to 1937 was not used to insert post-1935 work upon other sections, e.g. the use, in eelworm control, of chemicals to stimulate emergence from the encysted stage.

Chemical methods of plant protection come next under review, insecticides and fungicides occupying the greater part (210 pages) of the book. The chemistry of these materials is dealt with by Drs G. Hilgendorff and W. Fischer and the biological application by Drs W. Trappmann, W. Tomaszewski and A. Winkelmann. Each group is described systematically and all information of possible value to the applied biologist is assembled with full references. Upon controversial subjects, such as the chemistry of Bordeaux mixture or the action of the copper fungicides, each succeeding hypothesis is mentioned but mature criticism in the text indicates those of practical significance. The many misprints, especially in the titles of non-German articles, are irritating rather than misleading. As the citations appear to have been checked, it is difficult to understand how the spelling errors survived.

The comprehensiveness and richness of this section contrasts with the paucity of sign-posts to the information available. No index is provided, nor is the table of contents appearing in a separate advertisement leaflet helpful since it does not always coincide with the author's arrangement. Thus, derris and other pest control materials of vegetable origin are listed as subsection 7 of nicotine (p. 495) whereas the authors treat this group in section 6 (p. 504). Nor is good use made of the arrangement of the subject matter to facilitate reference. Insecticides and fungicides are classed as inorganic or organic, the inorganic materials being arranged upon the metal-metalloid —non-metal basis of the old-fashioned class books. A biologist trying to find the thallium rat poisons would never think of looking between the sections headed "Bleiverbindung" and "K-, Na-...Ce-Verbindung". Even the chemist would be lost among the organic compounds, arranged on no apparent plan. The most helpful approach is probably that based on function, a scheme which has been partly adopted in discussing supplementary materials. Finally, the device of systematic and distinctive headings has been poorly used. Thus, the reader might well think that the chapter headed "(b) Biologische Prüfung von Pflanzen- und Vorratschutzmitteln" was but a subsection of the previous subject "3. Farb-, Riech-,...Warnstoffe".

The criticism that it is difficult to use the book, in its present form, for purposes of reference has been stressed purposely since it is perhaps not too late to provide an index in which the chemicals are grouped according to use. The authors must be permitted full scope in the indexing of their sections for the preparation of the final part, with which a subject index is promised.

The biological methods of testing insecticides and fungicides are next discussed, in 20 pages, by Drs A. Winkelmann and H. Klinger. It is apparent that the subject matter employed by these authors does not go far beyond their laboratory at Dahlem.

Much of it has already appeared in the "Mitteilungen aus der Biologischen Reichsanstalt" and the present section is a useful supplement to Heft 55 of that publication. The opportunity for reviewing other methods and of displaying the value of biological assay in the analysis of the mode of action of insecticides and fungicides or in the development of new materials has not been taken. Surely the authors have heard of the Peet-Grady method, of Campbell's sandwich method, of the work of Tattersfield and his colleagues, to mention but a few of the many omissions concerning insecticides only. Even reference to their compatriot Gerhard Peters is not made although the authors must often have consulted his monograph *Chemie und Toxikologie der Schädlingsbekämpfung*. Nor is the interpretation of the results of biological tests referred to beyond the tabulation of empirical toxicity values of this type: 0 = no eating, I = traces of eating, etc.; space which could well be replaced by mention of the statistical methods due to Henderson Smith, Bliss, O'Kane and others.

The book closes with 15 pages of the section upon physical and chemical methods of testing pest control materials by Drs G. Hilgendorff and W. Fischer who adopt a welcome return to the completeness and comprehensiveness of earlier chapters. It is however disturbing to note that, whereas four closely printed pages are insufficient to describe the quantitative estimation of copper, but one short paragraph is devoted to surface tension, a property employed to evaluate the wetting and spreading powers of spray fluids. But without knowledge of the full contents of Part 3 of the volume, it would be dangerous to suggest that a lack of sense of proportion is apparent in this chapter.

H. MARTIN.

Third International Congress of Comparative Pathology, Athens, 1936. Reports, vol. I, part 2. Section of Plant Pathology. Pp. 1–274. Athens: Éditions "Flamma". 1936 (received July 1938).

The present part is devoted to the subject of "Immunity in Plants", and contains papers read to the Congress by Butler, Dufrenoy, Gäumann, Carbone, Magrou, Politis, Reed, Riker, Brenchley, Brooks, Fahmy, Rischkow, Salaman, K. M. Smith & Doncaster, Savulescu, Stakman & Hart, and Humphrey. In an *Extrait du volume des Comptes Rendus* (pp. 35), there are further papers on the subject by Boivin *et al.*, Cavadas, Pinoy, Sareganni & Cortzas, and Apostolides. The papers are in English, French, Italian, and German, conclude with a French résumé, and many possess bibliographies.

The papers cover a wide field: fungal, bacterial, and virus diseases; resistance of plants to poisons and alkalies; the relation of heredity, of nutrition, or of various chemical substances or physiological products to resistance; phagocytosis in plants. The work is marred by irritating misprints, e.g., on p. 85 the following variants of *Phyllosticta* occur: Phylosticta, Pyllosticta, Phallosticta. The papers form an excellent cross section of our present state of knowledge and should be a stimulus to further research on this intriguing and important subject.

WILLIAM B. BRIERLEY.

The Genus Septobasidium. By JOHN N. COUCH. Pp. ix + 480. University of North Carolina Press. 1938. $ 5.0.

All known species of *Septobasidium* occur on living plants in association with scale insects, and any fungus showing such relation is of interest to applied biologists, not only intrinsically, but because it may have importance in the field of biological control. When the author commenced his studies some 12 years ago about 75 species of *Septobasidium* had been named, but few had been described adequately or illustrated. In the intervening period Prof. Couch has added greatly to our knowledge of the genus and he has now assembled his studies in this imposing volume.

Ch. I (pp. 44) is a convincing and interesting account of the fungus-insect relationship based on the author's study of *S. Burtii* and certain other species. He concludes that "The fungus and insects live together interdependently; the fungus furnishes a home and protection for the insects, while, in return, the insects furnish food and a means of distribution for the fungus....The relationship here is, therefore, obviously one of symbiosis; both fungus and insects are benefited by the association at the expense of the tree." The author suggests that the form of the fungus is due to its response to a stimulus from the insects and is, perhaps, analogous to a "gall development". This chapter raises interesting problems for further study, e.g. why, when grown on artificial culture media, inappropriate living larvae, dead insects, etc., the fungus does not fruit or show any of the striking features so characteristic of its growth on the living scale insect.

In Ch. II (pp. 3), dealing briefly with pathological considerations and methods of control, the author concludes that although the damage to the trees may not be serious, "it is imperative that we look upon the *Septobasidium*-scale insect combination as distinctly harmful". Smearing kerosene emulsion paste on the patches of *Septobasidium* gave good results, but pruning of infected branches during the dormant season is regarded as the most effective means of control.

Ch. III (pp. 8) is devoted to the geographical distribution, host trees, and host insects of the fungus; Ch. IV (pp. 5) to structural features of taxonomic importance; Ch. V (p. 1) to hybridization in *Septobasidium*; and Ch. VI (p. 1) to its cytology. In nature the fungus seems rather prone to hybridization and as, apparently, it grows readily *in vitro* it would, could it be persuaded to reproduce, be a very interesting subject for genetical study. In Ch. VII (pp. 2), dealing with the relationships of the fungus, the author regards it "as a separate order on a par with the Auriculariales, Uredinales, and Ustilaginales". In Ch. VIII (pp. 2) the genus *Septobasidium* is described, and Ch. IX (pp. 10) contains an excellent key to the species. Ch. X (pp. 219) gives detailed diagnoses in English and descriptions of 170 species, and notes on three incompletely known species and on three excluded species.

The book is illustrated by a frontispiece, 60 text-figures, and a magnificent series of 114 plates. Sixty-six of these contain original drawings of microscopical features and the remainder original photographs of the fungus on host trees. There is a bibliography of 120 citations, and an index.

The work is a masterly production and ranks among the notable volumes in mycological literature.

WILLIAM B. BRIERLEY.

Soilless Growth of Plants. By CARLETON ELLIS and MILLER W. SWANEY. Pp. 155. 58 figs. New York: Reinhold Publishing Corp.; London: Chapman and Hall. $ 2.75 (13*s*. 6*d*.) net.

There is nothing new to botanists in the idea of growing plants without soil in nutrient solutions; many different methods employing water or sand culture have been used in scientific investigation of plant nutrition. It is, however, only in recent years that the possibility of using any of these methods for large-scale production of crops has been worked upon, principally in the United States. Two main lines have been followed; at Purdue University and the New Jersey Agricultural Experiment Station the growing of plants in mineral aggregates (sand, gravel, cinders or other material) has been tried on a commercial scale and found to give results with many crops at least as good as the normal methods of cultivation. In California, the work under Gericke has been directed to the commercial exploitation of liquid-culture methods, the so-called "tank culture". Reports of the successes achieved, especially in California, caught the fancy of journalists of the popular press, first in America and then in this country, and wild and fantastic stories were published of crop yields 400 and 500 % above normal.

The truth is, of course, that the methods may well have commercial advantages, but these lie, not so much in greatly increased yields, as in the greater degree of control over the nutrition of the crop and the presumably greater freedom from, or at least ease of control of, soil-borne diseases. The problem almost certainly is principally one of comparative cost, but in these days of need for expansion in food-producing power the idea is one which must be investigated.

The book under review is a clear and impartial account of the various methods which have been tried, or which might be tried, by the large-scale producer or the amateur. Full details with illustrations are given of the different types of apparatus and a range of suggested nutrient solutions is appended. An interesting chapter deals with the uses of the synthetic growth-substances and the chemical colchicine, recently shown by Blakeslee to have the remarkable property of inducing polyploidy in many plants. The book is one which should be read, not only by those interested in the practical problem, but by all workers on plant nutrition. They will find practical hints of great value.

<div align="right">R. H. STOUGHTON.</div>

Plant Growth Substances. By HUGH NICOL. Pp. xii + 108. London: Leonard Hill, Ltd. 1938. 3s. 6d. net.

The chief difficulty in reviewing this book lies in understanding to whom it is addressed. It is based upon a series of articles contributed by its author to *The Manufacturing Chemist* in 1937, from which one would infer that it was written mainly for chemists concerned either in the preparation or the identification and analysis of the substances. Certainly much more than half of the book would be unintelligible to anyone with no knowledge of organic chemistry. On the other hand chapters "for the layman" are introduced to give some idea of the practical uses to which synthetic growth substances have been, or may be, put. Unfortunately, for the chemist the chemistry is insufficient; to him the most valuable part will be the well-selected lists of references. The author admits frankly that he is not a physiologist, and the research worker in the field of plant response will seek in vain for the physiological implications and explanations of "hormone" action. For him, the chemical treatment will be of considerable value.

The author has, however, undoubtedly rendered service in collecting together so much information on the occurrence, sources, synthesis and identification of these substances, and in presenting it at a price so low that there can be no excuse for anyone who is even remotely interested in the subject not availing himself of that information.

It is unfortunate that, on p. 11, the author states that "at present...the only commercially available growth-substances the horticulturist and amateur gardener need trouble about, are *indole-acetic acid* and *phenyl-acetic acid*". Naphthalene-acetic acid and indole-butyric acid, as well as others, have been available for some time, certainly since before this book was written. The latter, at least, is probably a more useful compound than either of those mentioned, while the former is the most potent stimulator of root-initiation although, at the same time, having the greatest bud-inhibiting power. It may be noted that a commercial firm actually advertises five synthetic substances at the back of this very volume.

The author begins, apparently, in one chapter to attempt to clear up the vexed question of what general name should be used for these substances but, after seeming to favour the claims of Bottomley's term "auximone", he allows it to be inferred elsewhere in the book that he suggests the use of "growth-regulating" substance which he attributes to Eden but which was, in fact, used by Snow ten years ago.

As a reference book and source of information on the original (chemical) literature the book will be of real value to the research worker. It is feared that the "layman" will retire with a headache.

<div align="right">R. H. STOUGHTON.</div>

Common British Grasses and Legumes. By J. O. Thomas and L. J. Davies. Pp. viii + 124. London: Longmans, Green and Co., Ltd. 1938. 6s. 0d.

The importance to the agriculturist of a knowledge of the grasses is so generally recognized, that little need be said to enforce the value of another book. This book has been written for the farmer, schools, young farmers' classes and agricultural colleges and is exceptionally well illustrated. It is designed to supply, in concise form, a guide to the identification of the various species of British grasses and legumes. It is written in a simple, easy style and, for those who wish to use a technical book, it is one of the first that might be read with profit. The beginner often finds difficulty in distinguishing between the different species, but by using this book the student should learn the characters of the common forage plants with celerity. The main chapters deal with botanical descriptions of the more common grasses and legumes, arranged in alphabetical order of their species. The peculiarities of the structure of the various grasses are carefully explained and each species is illustrated by excellent line drawings. Two easily worked keys for the identification of the grasses and clovers by their vegetative characters are given. The authors have made the book intelligible to persons possessing little scientific knowledge: it also commends itself to those workers whose duty it is to educate the farming community.
J. S. L. WALDIE.

Plant Ecology. By J. E. Weaver and F. E. Clements. 2nd Edition. Pp. xxii + 601. London: McGraw-Hill Publishing Co., Ltd. 1938. 30s. 0d.

The first edition of this well-known American textbook was reviewed in the *Annals*, 1930, **17**, 398. In the intervening period the whole front of ecology has advanced, most strikingly perhaps, in methods of studying vegetation, and in knowledge of the fundamental units, of ecesis and invasion, of reactions and coactions, and in the study of the effects of light, wind and drought upon vegetation. The concept of xerophytism has greatly changed. The vast importance of climate and vegetation in soil development has been generally recognized, and the processes of plant succession, stabilization of climax vegetation, and the use of plants and plant communities as indicators, have become much clearer. These advances are reflected in the new edition which has largely been rewritten, and rearranged to follow a more logical sequence. The volume has been increased by 81 pages, the number of text-illustrations by 9 figures, and the bibliography by 430 citations. The book remains primarily American in outlook, with examples and illustrations drawn from American work and conditions, but it is one of the major landmarks in the literature of modern ecology.
WILLIAM B. BRIERLEY.

Forest Bibliography to 31st December 1933. Compiled and published by the Department of Forestry, University of Oxford. Part III, pp. 201–74. 1938. 12s. 6d.

Parts I and II of this *Bibliography* were reviewed in the *Annals*, 1938, **25**, 438. Part III covers C, Forest Protection, under the following headings: (1) Man. Demarcation, offences; (2) Animals, including bird and game preservation; (3) Atmospheric influences. Frost, insolation, wind, hail, snow, etc.; (4) Fire; (5) Weeds, including phanerogamous parasites, climbers, etc. (special, not included under Tending); (6) Other agencies: floods, swamps, shifting sands, avalanches, landslips, etc.; reclamation works, including protective afforestation; drainage; damage by chemical fumes; shelter-belts, shade trees, etc.; (7) Fencing, including hedges.
WILLIAM B. BRIERLEY.

Textbook of Dendrology. By W. M. HARLOW and E. S. HARRAR. Pp. xiii + 527. London: McGraw-Hill Publishing Co., Ltd. 1937. 25*s.* 0*d.*

An introductory section of 38 pages deals with nomenclature, classification, identification, variation, and description of species: a section of 193 pages is devoted to the Gymnosperms; and the remainder of the book to Angiospermous trees. There are a short glossary, 11 pages of selected references, and an index. Each plant family is characterized briefly, and a tabular conspectus of native genera given. Generic characters are described, and the several species receive consideration under the headings: distinguishing characters, general description, range, botanical features. There are 224 text-illustrations, each containing several figures, of botanical features of the more important species. The book covers the important forest trees of the United States and Canada. The American code of nomenclature is used but, where they differ, international code names are given in italics. The book is a useful compendium and, in addition to the immediate data, contains some generally interesting botanical matter and occasional notes on fungal diseases and insect pests.

<div align="right">WILLIAM B. BRIERLEY.</div>

Cryptogamic Botany. By GILBERT M. SMITH. Vol. I: Algae and Fungi. Pp. viii + 545. 24*s.* 0*d.* Vol. II: Bryophytes and Pteridophytes. Pp. vii + 380. 18*s.* 0*d.* London: McGraw-Hill Publishing Co., Ltd. 1938.

These two volumes contain an excellent general survey of the classification and morphology of the Cryptogams; more physiological and applied aspects are excluded. They are suitable for students reading for a general degree in botany, and the briefly annotated bibliographies, containing about 2000 references, will be useful for honours students and research workers. The consideration of the various groups is largely on the basis of representative American types and series, but most of the examples are widely spread and available to European workers. The author's expectation "that some botanists will disagree with the allocation of space, especially in the relative proportions devoted to the algae and to the fungi" is likely to be realized since the algae receive 339 pages and the fungi 147. Furthermore, the treatment of the fungi seems to me to be less satisfactory than that of the other major groups. A notable feature are the 523 rather beautiful text-illustrations many of which have been drawn specially for the book. On the other hand there are a number of rather irritating misprints, particularly in the spelling of generic and specific names. The work is a fine achievement and a valuable addition to cryptogamic literature.

<div align="right">WILLIAM B. BRIERLEY.</div>

Evolution. Essays on Aspects of Evolutionary Biology presented to Prof. E. S. Goodrich on his Seventieth Birthday. Edited by G. R. DE BEER. Pp. viii + 350. With a frontispiece and 2 plates. Oxford: Clarendon Press. 1938. 15*s.* 0*d.*

The presentation of a "Festschrift" by the colleagues and pupils of a distinguished man on his attainment of 70 years is a pleasing custom, and the recipient's pleasure must be increased when the volume lies in a field in which he has always been deeply interested and to which he has made notable contributions.

The Preface states that the book covers "as evenly as possible the various more important aspects of modern knowledge concerning evolution", but this is something of an exaggeration since except for J. B. S. Haldane's essay on "The nature of inter-

specific differences" and that by H. G. Thornton on "Bacterial strains and variation", plants and plant evolution are almost unmentioned. The nineteen essays are all interesting and well written, although certain of them are, perhaps, a little thin. The subjects dealt with cover a wide range, but in addition to the two already mentioned the essays which may be of interest to applied biologists are the following: "Insect adaptation as evidence of evolution by natural selection" by Sir Edward Poulton: "The genetic basis of adaptation" by E. B. Ford: "The formation of species" by O. W. Richards: "Life-cycles of certain infusoria with observations on specificity in parasitic protozoa" by Helen P. Goodrich: "Helminths and evolution" by H. A. Baylis.

The book closes with a bibliography of the scientific works of Prof. E. S. Goodrich, an index, and a list of subscribers.

<div align="right">WILLIAM B. BRIERLEY.</div>

Heredity. By A. FRANKLIN SHULL. 3rd Edition. Pp. xvii + 442. London: McGraw-Hill Publishing Co., Ltd. 1938. 21s. 0d.

The chapter headings run as follows: Development of knowledge of genetics: Fundamental structure of organisms: Production of new cells: Origin of new individuals: Development of new individuals: Mechanism of heredity: Simplest phenomena of heredity: Dominance: Backcross and testcross: Sex-linkage: Multiple alleles: Lethal genetic characters: Two or more independent pairs of genes: Interaction of genes: Modified F_2 ratios: Modification by environment: Chance and heredity: Linkage: Proof that genes are in chromosomes: Non-Mendelian inheritance: Determination and development of sex: Heredity and evolution: Inheritance of human structural characters: Human heredity, physiological characters: Inheritance of mental characters: Practical applications of heredity: Eugenics: The population problem: Race problems: Immigration. There are also a statistical appendix, questions and problems on the various chapters, a selected bibliography of 269 references, and an index.

The book is a good general introduction to heredity, illustrated mostly by animal examples, and with human heredity emphasized throughout. The usual sequence in general texts on this subject is the presentation of experimental results followed by their cytological interpretation, but the author's teaching experience has led him almost to reverse this sequence. The book is a well written and balanced exposition, and even in the later chapters the author usually does not go beyond his evidence.

<div align="right">WILLIAM B. BRIERLEY.</div>

STUDIES IN FRUIT STORAGE

I. INFLUENCE OF THE STAGE OF MATURITY AND STORAGE TEMPERATURE ON RESPIRATORY DRIFTS DURING THE RIPENING OF TOMATO FRUITS

By B. N. SINGH and P. B. MATHUR

From the Institute of Agricultural Research, Benares Hindu University, India

(With 6 Text-figures)

CONTENTS

INTRODUCTION

INVESTIGATIONS in connexion with the storage of fruits by various workers have shown that the storage life of fruits can be prolonged considerably by suitable combinations of the three factors, temperature, carbon dioxide and oxygen concentrations in the storage room. With certain exceptions, low temperatures, high carbon dioxide concentrations and low percentages of oxygen depress respiratory activity in living tissue and thus retard the rate of physiological shrinkage during the storage of fruits. On the basis of experience in various storage investigations, a method has been developed which involves the storage of fruits at comparatively low temperatures with fairly high concentrations of carbon dioxide in association with low percentages of oxygen: this is designated the "refrigerated gas-storage" of fruits.

In the tropics, however, humidity is a factor of as great importance as any of those above mentioned. Weight losses due to low relative humidity can be checked more or less effectively by a suitable combination of high humidity and low temperature. It may be noted that atmospheres of the same relative humidity will have lower evaporating powers the lower the temperature. Among the other factors conditioning

the duration of storage life of fruits are variety, manurial treatment, maturity at picking, and diseases during storage.

The outlook for long-term storage of tomato fruits is not encouraging, but by attention to variety, manurial treatment, stage of maturity at picking and the environmental conditions of the fruits in the storage room, it is possible to extend greatly the time of marketing and to facilitate the long-distance transport of tomato fruits.

In this preliminary report the influence of two factors only, namely, the stage of maturity at picking and the storage temperature, on storage life and loss of weight during the storage period will be considered.

LITERATURE

According to Wright *et al.* (1931) the tomato fruit tends to be intolerant of a severe retardation in its respiratory process by temperatures near 0° C. and to break down rapidly upon removal to higher temperatures after only short exposures to low temperatures.

In the temperate regions, Barker (1927) and Wright *et al.* (1931) have advocated storage temperatures in the range 10–15° C. for field-grown tomatoes, though somewhat lower temperatures appear to be permissible for tomatoes grown in the tropics.

Seasonal factors during the growth of the fruit are important in determining the storage life of tomato fruits. For example, Barker (1927) found that temperatures below 15·5° C. were injurious to English hothouse tomatoes, whereas the investigations of Wardlaw & McGuire (1932) indicate that tropically grown tomatoes are stored best at about 5° C.

Kidd & West (1932) found that English summer-grown fruit resembled the tropical fruit in physiological behaviour during storage, whereas the fruits grown in autumn were found to be intolerant of temperatures below 15° C.

Walford (1938) has recently published data dealing with the influence of season and the stage of maturity at picking upon certain aspects of the physiology of the fruit held at 12·5° C. during storage. Season was found to have a marked influence on the physiology of the fruit. He concluded that, while ripening on the plant, fruits pass through the senescent rise of respiration irrespective of the time of year in which they are grown in the greenhouse. In the summer fruit the senescent rise is evident in isolated fruits kept at 12·5° C., and the fruits are characterized by a lack of durability normal to this fruit. But in the winter fruit, if picked at maturity and still green, departures from the conventional type are obtained. The respiration record reveals an extended period of remarkably

steady rate while ripening is in progress and for long afterwards, suggesting a metabolically stable condition quite different from that obtained in the fruit of the conventional type.

MEASUREMENT OF RESPIRATION

The fruits (var. Abundance) for this investigation were obtained from a tomato plot fertilized with 300 lb./acre of ammonium sulphate, 114 lb./acre of double superphosphate and 70 lb./acre of potassium sulphate.

After being picked from the vines, the fruits were brought immediately to the laboratory, the calyces were removed, the injured portions waxed and the weight of each individual fruit recorded. The fruits were kept in glass respiration chambers and the rate of respiration estimated by the Pettenkofer tube method. The absorption periods ranged between 24 and 48 hr. The respiration records represented in this paper refer to single fruits. Such a procedure is essential in connexion with studies on ripening of fruits, as individual variations are often masked if fruits of different developmental stages are put in the same lot for experimentation.

RESULTS

The growth curves (Figs. 1, 2) as obtained by plotting successive fresh as well as dry weights against time show a slow increase in the beginning, followed by a very rapid increase which in turn ends by a gradual decrease and final cessation of growth. In Figs. 1 and 2, A and D refer to second trusses, B and E to fourth, and C and F to sixth trusses of two completely manured tomato plants. Considering the dry weight as the criterion of growth, the highest weight is attained by the fruits of the sixth truss (counting from the base) followed by that of the second and fourth trusses.

Fig. 3 records the respiration intensities and percentages of dry matter in fruits picked at different stages of development from the basal trusses of six tomato plants. The percentage of dry matter is highest in the young fruits, then declines and thereafter remains fairly constant throughout maturity and ripening. Respiration intensity is also highest in the young fruits and declines with further growth of the fruits. During the colour change from orange to red which is characteristic of ripening in tomato there is again a rise in respiration. This peak value has been described by the English plant physiologists as the "climacteric".

The data plotted in Fig. 4 have been obtained by following respiratory drifts of individual fruits picked at various stages of ripening (on the

basis of colour) and placed in storage at fairly constant temperatures of 14·7, 9·8 and 5·7° C. with adequate aeration. A significant point brought out by Fig. 4 is that irrespective of the stage of ripening at picking and the temperature of storage, winter field-grown tomatoes pass the
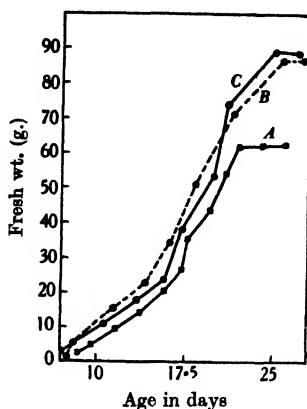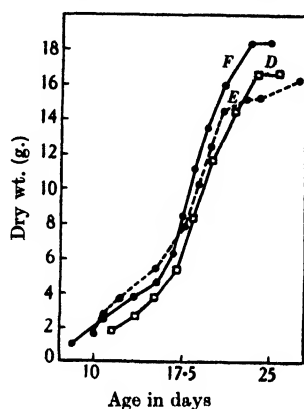
Fig. 1.   Fig. 2.

Figs. 1 and 2. Growth curves of tomato fruits constructed on the basis of fresh, as well as dry, weights. *A* and *D* refer to second trusses, *B* and *E* to fourth, and *C* and *F* to the sixth trusses of two completely manured tomato plants.
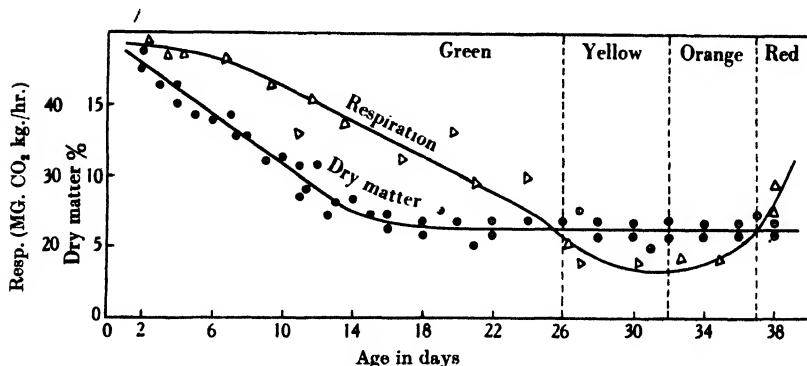
Fig. 3. Respiration intensities and percentage dry matter of fruits picked at different stages of development from the basal trusses of six completely manured tomato plants.

climacteric stage sooner or later. In Fig. 4 *green* refers to fully grown fruits having a green colour turning yellow, and *yellow* and *orange* refer to fruits picked in the yellow and orange stage respectively. A significant point observed was that certain tomatoes picked in the green stage showed

no evidence of the occurrence of the climacteric peak value during storage, though the usual sequence of colour change was recorded. One such case is shown graphically in Fig. 4 by bold dots. In this connexion it is interesting to note that Walford (1938) obtained different types of
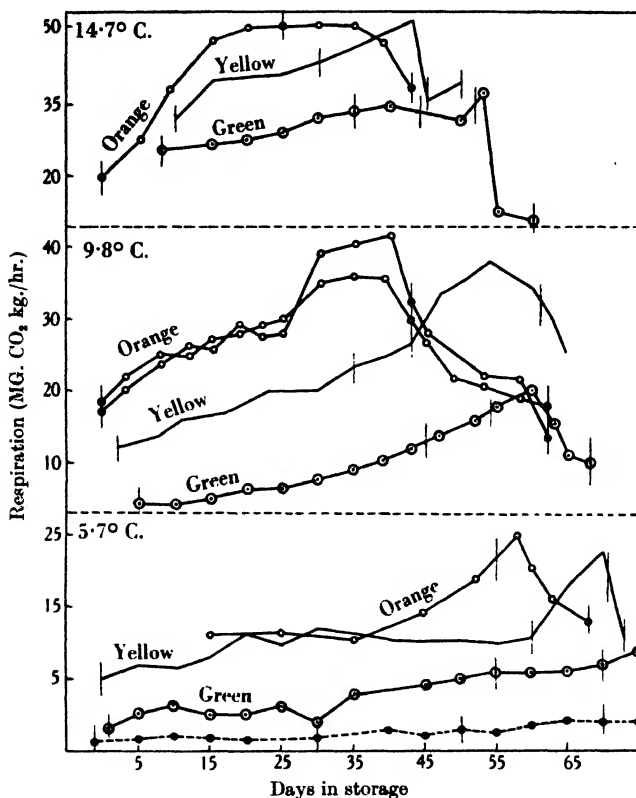
Fig. 4. Respiratory drifts during storage of green, yellow and orange fruits obtained from the basal trusses of four completely manured plants. The change of colour during storage is indicated by vertical lines crossing the curves, the order of the change in colour being green, yellow, orange, red. The colour at the commencement of the storage period is indicated on each curve.

respiration records with summer- and winter-grown tomatoes which he has designated as the "conventional" and the "anomalous" types. The autumn-winter-early-spring fruit, if picked approximately mature green, departs from the normal type. The respiration record reveals an extended

period of remarkably steady rate of metabolism and the storage life of the fruit is considerably increased.

Table I records the mean storage life in days and the mean physiological loss in weight during storage of fruits picked in the green, yellow and orange stages and placed in storage at 14·7, 9·8 and 5·7° C. The storage life of fruits is the longest at 5·7° C., followed by those stored at 9·8 and 14·7° C. At a given temperature the storage life is the longest in the case of green fruits and decreases progressively with increasing degrees of ripening of the tomatoes at the time of storage.

Table I. *Storage lives and physiological losses in weight of tomatoes stored at various temperatures*

Colour of the fruit at storage	Mean storage life days	Mean % loss in weight
Storage temp. 14·7° C.		
Green	60	12·7
Yellow	50	12·1
Orange	43	12·0
Storage temp. 9·8° C.		
Green	68	12·0
Yellow	65	11·8
Orange	62	11·6
Storage temp. 5·7° C.		
Green	75	10·7
Yellow	73	10·7
Orange	68	10·2

When tomatoes are transferred from cold storage to higher temperatures for marketing, the excessive rate of respiration following this change of environment usually results in a more or less complete breakdown of the fruits. Experiments were, therefore, conducted to investigate the influence of a period of cold storage (20 days) at 4, 7, 10, 13 and 16° C. on the rate of respiration of green tomatoes on transference to 20° C. Relevant data obtained are shown graphically in Fig. 5. In general, when tomatoes are raised from lower to higher temperatures, the output of carbon dioxide at the higher temperature is temporarily increased above the level that it subsequently attains at that temperature. Fig. 5 further indicates that the lower the storage temperature the higher the initial respiration rate and the longer the time necessary for it to come to equilibrium at the higher temperature. After the attainment of the peak value at the higher temperature the rate of decrease is the greatest in the tomatoes stored at the lowest temperature, namely, 4° C.

More or less similar results were obtained with green tomatoes that had been in storage at 4° C. for 10 days and were transferred thereafter
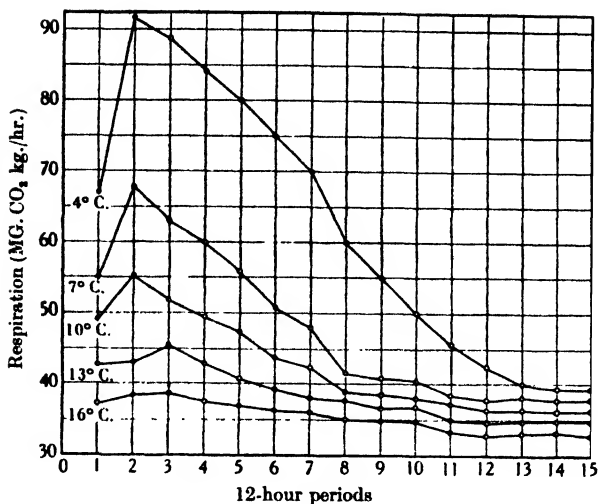
Fig. 5. Respiration rates in tomatoes from different storage temperatures (indicated on the curves). The respiration measurements were made at 20° C.
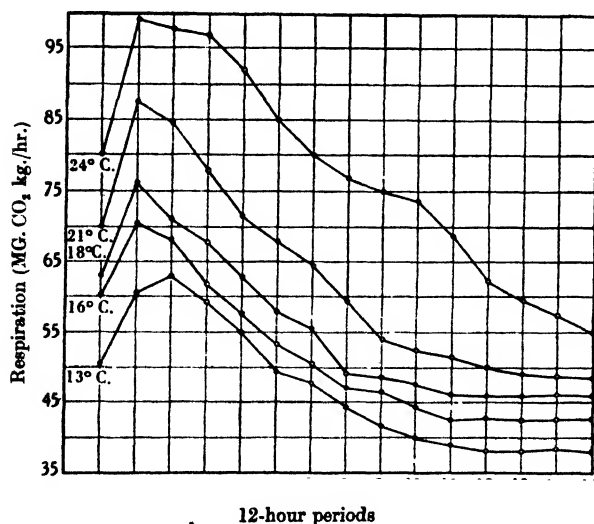
Fig. 6. Respiration rates in tomatoes from 4° C. storage. The temperatures given on the curves are those at which respiration determinations were made.

Studies in Fruit Storage

to 13, 16, 18, 21 and 24° C. for respiration determinations (Fig. 6). The initial high rate of respiration varies with the temperature at which the respiration determinations are made. The peak value of respiration intensity was obtained in 24 hr. in fruits whose respiration was measured at 16, 18, 21 and 24° C., whereas in tomatoes kept at 13° C. for respiration determinations the peak value occurred after 36 hr.

The values of R.Q. obtained on comparable lots of tomatoes by means of a gas-analysis method (Singh & Mathur, 1936 *b*) in both the series of experiments are presented in Table II. Generally speaking, the value of

Table II. *Values of* R.Q. *of tomatoes at successive 12 hr. periods after transference to higher temperatures*

Storage temp. (°C.)	Temp. (°C.) at which resp. was measured	12 hr. periods													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
4	13	1·16	1·17	1·14	1·11	1·11	1·09	1·08	1·07	1·09	1·06	0·99	1·00	0·99	1·00
4	16	—	1·14	1·12	1·11	—	1·09	1·08	1·09	1·08	1·09	0·99	1·01	1·01	1·02
4	18	1·19	1·12	1·12	1·17	1·17	1·11	1·11	—	—		1·00	0·99	0·99	1·02
4	21	1·17	1·13	1·13	1·27	1·20	1·17	1·11	1·09	1·07	1·08	1·02	1·00	0·98	1·01
4	24	1·21	1·29	1·31	1·30	1·21	1·05	1·04	1·03	0·99	1·07	1·08	1·00	0·99	0·99
4	20	1·22	1·23	1·27	1·20	1·20	1·19	1·17	1·16	1·17	1·16	1·09	1·01	1·00	1·00
7	20	1·20	1·20	1·26	—	—	—	—	1·17	1·09	1·08	1·07	1·01	1·00	1·00
10	20	1·19	1·17	1·22	1·19	1·17	1·11	1·15	—	1·06	—	·—	0·99	1·01	1·01
13	20	1·19	1·20	1·20	1·16	1·16	1·12	1·14	—	1·05	1·05	1·05	0·98	1·02	1·03
16	20	1·18	1·19	1·19	1·10	1·11	1·12	1·17	—	1·05	1·05	1·05	1·01	1·03	1·00

R.Q. is considerably higher than unity immediately after transference to higher temperatures and drops down to approximately unity after 96–108 hr. A significant point observed in connexion with the values of R.Q. is that the R.Q. attains unity after 96–108 hr., but the rate of respiration is observed in certain cases, particularly in the fruits belonging to 4° C. storage, to be rapidly falling even after 144 hr.

The respiration curves obtained in fruits after transference from a lower to a higher temperature are therefore divisible in two parts: (i) high R.Q. associated with a rapidly falling respiration intensity, and (ii) a value of R.Q. equal to 1·0 accompanying either a falling respiration intensity or a level phase of the rate of carbon dioxide output depending upon the temperature of storage and the temperature at which the respiration determinations are made.

The first aspect of the problem, namely, the association of a high R.Q. with a rapidly falling respiration is probably attributable to (i) anaerobiosis, due to a rapid transference from low to high temperature, (ii) temporary flush at the higher temperature of carbon dioxide held in the sap and the atmosphere of the intercellular spaces during storage at

low temperatures, and (iii) accumulation of substances at low temperatures that give out carbon dioxide at higher temperatures without a proportionate intake of oxygen. In this connexion it may be noted that Singh & Mathur (1936a) have observed that considerable amounts of carbon dioxide accumulate in tomato fruits during ripening.

The second aspect of the problem, namely, values of R.Q. approximating to unity accompanying falling respiration intensity, is explicable on the basis of the assumption that respirable substances accumulate at low temperatures. In this connexion it is interesting to note that an accumulation of sucrose and reducing sugars usually occurs in potato tubers held at low temperatures (Hopkins, 1924; Barker, 1936).

Summary

1. The growth curves obtained by plotting successive fresh as well as dry weights are of the usual sigmoid type. The percentage of dry matter is the highest in young fruits and thereafter declines and remains fairly constant during maturity and ripening of fruits. The respiration curve during the growth of the fruits shows two high values separated in time: one is initial and represents a high rate of respiration in young fruits, while the other occurs at the onset of senescence.

2. Irrespective of the stage of ripening at picking, winter-grown tomatoes show the usual "climacteric" rise during the colour change from orange to red. In certain tomatoes there was no evidence of the occurrence of the climacteric, though the sequence of colour change from green through yellow and orange to red was of the usual type.

3. The storage life of the fruits is longest at 5·7 followed by 9·8 and 14·7° C. At a given temperature the storage life is longest in fruits picked green as compared to those picked in the yellow and orange stages.

4. The respiration curves obtained in fruits after transference from low to high temperatures are divisible into two parts: (i) high R.Q. associated with a rapidly falling respiration intensity, and (ii) a value of R.Q. approximating to unity accompanying either a falling or a steady respiration rate.

References

BARKER, J. (1927). Cold-storage trials with tomatoes. *Rep. Food Invest. Bd, Lond.*
—— (1936). Analytical studies in plant respiration. VI. The relation of the respiration of potatoes to the concentration of sugars and to the accumulation of a depressant at low temperatures. Part 3. The relation of the respiration to the concentration of sucrose. *Proc. roy. Soc.* B, **119**, 453.

HOPKINS, E. F. (1924). Studies in potato storage. Relation of low temperature to respiration and carbohydrate changes in potato tubers. *Bot. Gaz.* **78**, 311.

KIDD, F. & WEST, C. (1932). Low-temperature tolerance of summer-grown and autumn-grown hothouse tomatoes. *Rep. Food Invest. Bd, Lond.*

SINGH, B. N. & MATHUR, P. B. (1936 a). Dissolved carbon dioxide and the ripening of tomatoes. *Nature, Lond.,* **137**, 704.

—— —— (1936 b). An adaptation of Haldane's gas-analysis apparatus. *New Phytol.* **35**, 418.

WALFORD, E. J. M. (1938). Studies of the tomato in relation to its storage. I. A survey of the effect of maturity and season upon the respiration of green-house fruits at 12·5° C. *Canad. J. Res.,* Sec. C, **16**, 65.

WARDLAW, C. W. & McGUIRE, L. P. (1932). The storage of tropically grown tomatoes. *E.M.B. Bull.* no. 59. [See also WARDLAW, C. W. (1937). Tropical fruits and vegetables: an account of their storage and transport. *Imp. Coll. Trop. Agric., Trin., B.W.I.; Low Temp. Res. Sta. Mem.* no. 7. Bibliography cites 64 references of papers dealing with the tomato.]

WRIGHT, R. C., PENTZER, W. T., WHITEMAN, T. M. & ROSE, D. H. (1931). Effects of various temperatures on the storage and ripening of tomatoes. *Tech. Bull. U.S. Dep. Agric.* no. 268.

A CANKER AND DIE-BACK OF ROSES CAUSED BY *GRIPHOSPHAERIA CORTICOLA*

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(With Plates XIV and XV)

CONTENTS

INTRODUCTION

In January 1934 Dr G. E. Deacon of Brundall, Norwich, sent to the senior author some green stems of Kokulensky's *canina* rose stock bearing sunken brown lesions with a purplish margin (Pl. XIV, fig. 1). The lesions were ½–¾ in. long and extended about half-way round the stem; some were obviously the sites of rust attack (*Phragmidium mucronatum*), but others were not. On the dead bark there were numerous small, brownish black fructifications containing three-septate, light brown spores formed at the extremities of slender hyaline conidiophores; the basal cell of the spores was usually colourless. The fructifications were either acervuli or irregularly shaped pycnidia with an ill-defined ostiole. The fungus clearly belonged to the genus *Coryneum* or *Hendersonia*, several species of which have been recorded on rose stems. A culture of the fungus was established in order to test its pathogenicity. Further material of the disease was sought, and in addition to the first isolation the following cultures were established from roses:

No. 2: from a dead stem of Colchester *canina* rose stock sent by Dr Deacon, February 1934.

No. 3: from a dead stem of *Rosa Moyesii* collected by Dr d'Oliveira in the Cambridge Botanic Garden, March 1934.

No. 4: from a lesion on a green stem of an unknown cultivated variety sent by Dr Deacon, April 1934 (Pl. XIV, fig. 2).

No. 5: from a canker on a green stem of "Isobel" rose collected at Wisley, June 1934.

Nos. 6 and 7: from lesions on green stems of Kokulensky's *canina* rose stock collected near Norwich, December 1934.

No. 8: from a dead stem of an unknown cultivated variety, March 1935.

Nos. 9 and 10: from dead stems of unknown cultivated varieties sent by Dr Deacon, March 1935.

No. 11: from a canker on "Gloria Mundi" rose growing under glass at Keynsham, sent by Mr L. Ogilvie, Long Ashton, Bristol, March 1936.

In addition, the same disease was sent to us by Mr G. Samuel on "Karen Poulsen" roses from his garden at Harpenden and by Mrs M. Gregor from cultivated roses at Crieff, Scotland.

SYMPTOMS OF THE DISEASE

At the Field Station of the Cambridge Botany School, where there is a large collection of roses for experimental purposes, the disease was established by inoculation, after which it was allowed to spread spontaneously. The disease is confined to the stems and does not directly affect the leaves. It appears either as a canker or a die-back. The cankers begin to develop as brown depressions in the green bark, frequently around a dead bud, a thorn scar, a rust lesion or some other kind of injury. The margin of the canker is smooth and purplish in colour. The cankers usually extend with time to about half-way round the stem and they may be half an inch to several inches in length. As the bark dies numerous small blackish fructifications develop. After some time the canker usually ceases to spread; the bark becomes sunken, but the wood is not exposed and the margin does not become rough and swollen as it commonly does in the canker caused by *Coniothyrium Fuckelii* Sacc. In the young stages, however, the two kinds of cankers are hardly distinguishable unless the parasites are fruiting. Occasionally, the cankers caused by the disease now under consideration completely girdle the stem, when the upper part dies back. Alternatively, the fungus may infect a pruning wound and spread downwards extensively, again causing die-back. If cankers or stems killed by the fungus are allowed to remain on a susceptible variety of rose, the disease spreads somewhat rapidly on account of secondary infections from spores produced in the first lesions. Under these conditions "die-back" of the bushes becomes conspicuous.

CULTURAL STUDIES

Single spore cultures were established from each of the isolations nos. 1–11 and were grown side by side on a variety of agar media such as Dox, potato dextrose, malt extract, oatmeal and rose stem extract, and also on sterilized stems of *Rosa canina*. The cultures spored best on Dox's medium and on rose stems, so the various isolations were maintained on these throughout the investigation. Subsequently, Miss A. E. Jenkins kindly sent two cultures (nos. 66 and 67) of *Coryneum microstictum* B. and Br. from roses in the United States, but only no. 66 spored freely.

On Dox's medium the cultures, including Miss Jenkins' no. 66, produced varying amounts of white aerial mycelium and dark spore aggregates which differed somewhat in abundance and colour in the respective isolations. The surface of the medium was sometimes practically covered with the spore masses, almost to the exclusion of aerial mycelium. Pl. XV, fig. 3, shows cultures of four of the isolations on Dox's agar. These spore aggregates arose directly from massed conidiophores: they were not formed in pycnidia nor was there a compact layer of hyphae below them as in a typical acervulus. There were minor differences in the shape, size and septation of the spores in the various isolations, which had been previously noted on the natural host. These differences will be referred to later.

On growing isolations nos. 1–10 on Dox's medium at different temperatures their growth rates were found to be essentially the same. the optimum temperature being about 20° C. Little growth occurred at 5 or 30° C., and isolation no. 4 did not grow at all at the latter temperature. Temperatures above 20° C. favoured the development of aerial mycelium to the detriment of spore formation.

On sterilized rose twigs the various isolations showed similar slight macroscopic differences. The conidial pustules on the surface of the twigs were built up in the same way as on Dox's medium. Typical pycnidia or acervuli were not formed in the bark. After about 3 months, however, isolations nos. 1, 5 and 6 gave rise to perithecia in the bark, the ascospores of which were three-septate and hyaline. The perithecia were first seen in culture in February 1935. The formation of perithecia enabled the fungus to be identified as *Griphosphaeria corticola* (Fckl.) v. Höhnel. Single ascospores were isolated and grown in culture: the same conidial stage was again produced. Soon after perithecia were seen in culture they were found on rose stems inoculated in the field with these

particular isolations. Perithecia have not been seen in connexion with the other conidial isolations, either in the field or in culture.

The conidia germinate readily in water and in nutritive media, each cell, except generally the hyaline basal one, forming a germ tube. Germination commences within a few hours at 20° C. The ascospores also germinate readily and each cell may form a germ tube somewhat stouter than that from a conidium. The ascospores occasionally germinate inside the ascus by the formation of germ tubes, when the ascus wall gradually breaks down. Germination of ascospores within the ascus to form sporidia, as observed sometimes by Petrak (1921), has not been seen.

It will be convenient here to describe the method of ascospore discharge from the perithecium. At maturity and under moist conditions the asci elongate successively so that the tips protrude slightly beyond the ostiole. An ascus, so extended, discharges its spores violently and then contracts, its place being taken by the next ascus ready to liberate its spores, and so on.

INOCULATION EXPERIMENTS

A preliminary test of the pathogenicity of isolations nos. 1–5 was carried out during the summer of 1934 with the following varieties of rose bushes: Etoile de Hollande, Shot Silk, Lady Inchiquin, Golden Emblem, Independence Day, Duchess of Atholl, Mrs Sam McGredy, Mrs G. A. Van Rossen, Mabel Morse, Angele Pernet, Madame Edouard Herriot, Lady Pirrie, Ophelia, Madame Butterfly, Mrs Henry Bowles and Mrs A. R. Barraclough. T-shaped wounds were made in the bark of 2-year-old stems, and a culture of the fungus from Dox's medium was inserted under moist conditions. Within 6 months incipient cankers had been formed on several varieties by each of the isolations; on some of the cankers conidial fructifications had already developed on the dead bark, from which cultures were again established. As it was evident that all the isolations were pathogenic to roses many series of inoculations were subsequently carried out under more natural conditions and at different times of the year.

During November 1934 2-year-old stems of the above varieties were cut back and the exposed extremities inoculated with an aqueous suspension of conidia of isolations nos. 1–5, such wounds being similar to those made in pruning. It had already been ascertained that on applying a spore suspension to the cut extremity of a rose twig the spores were sucked a considerable distance into the wood vessels, as described by Brooks & Moore (1923) for *Stereum purpureum*. Some of the stems

inoculated in this way were left exposed to the air, while others were covered with cotton-wool which was kept moist for a week following inoculation. Adequate controls were maintained. In January 1935 it was evident that most of the inoculated stems were being successfully invaded, whether exposed or protected. Browning of the bark occurred from the cut end downwards, there frequently being a purplish zone at the junction of diseased and healthy tissues. At this date no change was detected in the controls. By May 1935 some of the inoculated stems had died back to the extent of 6–12 in. and nearly all were bearing conidial fructifications on the dead bark. A few of the inoculations were rendered worthless through secondary infection by *Botrytis cinerea*, as were also some of the control experiments. Apart from infection by *Botrytis*, however, the control stems had died back at the most only to the nearest bud. All the isolations nos. 1–5 again proved to be pathogenic, and to judge by the amount of die-back nos. 1 and 5 were the most lethal. In this experiment, as in several others, the varieties Golden Emblem, Duchess of Atholl and Lady Inchiquin were the most susceptible, and Independence Day, Mrs G. A. Van Rossen and Madame Edouard Herriot the most resistant, although no variety was entirely unaffected. In addition to conidial fructifications, perithecia of *Griphosphaeria corticola* were found in May 1935 on stems inoculated with isolations nos. 1 and 5. In connexion with these inoculations it was noticed that, in general, the disease ceased to progress during the summer following inoculation; once invasion had been stopped the disease did not subsequently extend.

A similar experiment carried out in November 1935 with isolations nos. 6, 7, 8 and 10, showed that these also were pathogenic, isolation no. 6 being the most active and resulting in the formation of perithecia of *Griphosphaeria corticola* as well as conidia.

It was then decided to ascertain whether infection of the cut ends of shoots was more likely to occur at some seasons of the year than at others, as described by Brooks & Moore (1926) for *Stereum purpureum*. Isolations nos. 1 and 5 were chosen for this series of experiments as being most pathogenic. Freshly cut stem extremities of the sixteen varieties of roses were inoculated with aqueous suspensions of conidia during January, March, May, July, September and November. Except for the inoculations made in July nearly all were successful, observations on the stems being continued until the end of the summer following inoculation. Most of the stems inoculated in July were not appreciably affected at all, but a few became invaded to the extent of about an inch. Several of these July inoculations were cut off and examined; sections showed that the

browning of the internal tissues associated with the progress of the fungus was intensified at the lower limit of invasion as if a "gum barrier" had prevented further spread of the disease. In the completely unsuccessful inoculations the "gum barrier" was situated just below the exposed end of the stem. Other evidence showed that the spores of the inoculum had germinated, but that advance of the mycelium was soon stopped by the reactions of the host tissues at this time of year. These experiments showed that it was virtually impossible for the fungus to cause infection during the middle of the summer when inoculation was carried out in a natural manner. With the November and January inoculations invasion was very slow until the rise in temperature in spring. Again, it was observed that further dying back of the stems ceased during the summer following inoculation, although considerable lengths of stem might have been killed before then.

Some evidence was obtained that if freshly cut ends of stems were left exposed for 3 months before inoculation it was difficult to bring about infection whatever the time of year. Unfortunately, however, the largest experiment designed to test liability to infection after varying periods of exposure was ruined by a severe attack of *Botrytis cinerea* which caused extensive die-back.

Another experiment was planned to determine whether the age of the tissues influenced invasion. Young, incompletely hardened stems and stems 1 and 2 years old were cut back and the exposures inoculated with a conidial suspension of isolation no. 1. Infection almost invariably occurred, and there was no indication that the age of the tissues appreciably influenced susceptibility. Natural infection, however, has not been seen in stems less than about 1 year old.

Infection through pruning wounds was obviously one channel of invasion, but in the naturally occurring specimens of the disease it had been noticed that infection sometimes centred round rust lesions, dead buds and thorn scars. Attempts were therefore made to ascertain whether infection could be induced experimentally through such injuries and also through leaf scars and wounds made by thorns. The varieties Golden Emblem, Duchess of Atholl, Mrs Sam McGredy, Lady Inchiquin and Etoile de Hollande were used for this purpose. As there were no rust lesions on the stems of the cultivated varieties stocks of *Rosa canina* affected by rust were used in this connexion. The dead buds, which were inoculated in April 1936, had been killed by frost shortly before then. Thorns were removed and the scars forthwith inoculated. Other wounds were deliberately made by scratching stems with the thorns of others,

thus simulating a type of injury which is common in Nature. All these kinds of injuries and some leaf scars were inoculated with an aqueous suspension of conidia of isolation no. 1, adequate controls being maintained. In due course characteristic cankers developed around the dead buds, rust lesions and wounds made by thorns, which had been inoculated with the fungus. Most of the inoculated thorn scars and a few of the leaf scars were similarly affected. The controls remained healthy. It is clear, therefore, that there are many ways in which this disease of roses can become established. With regard to infection through rust lesions it is of interest that Deacon (1938) has described infection by *Coniothyrium Fuckelii* through the same channel.

Certain kinds of rose stocks also proved to be susceptible to the disease. Only one series of experiments with stocks will be described, which concerned the following: *Rosa canina* (from cuttings), Kokulensky's *canina* (from seedlings), *R. multiflora polyantha* (from seedlings), *R. rugosa* (from cuttings), *R. rubiginosa* (from seedlings) and Manetti (from cuttings). Stems of these stocks were cut back in November 1935 and inoculated, as previously described, with conidial suspensions of isolations nos. 1 and 5. In the late spring of 1936 most of these inoculated stems had died back to the extent of about two inches and bore conidial pustules. *R. canina* and Kokulensky's *canina* were somewhat more susceptible than the others, but progress of the disease was not so rapid as in cultivated varieties of roses.

All the inoculations so far described were made with mycelium or conidia of the various isolations. Ascospores of *Griphosphaeria corticola* were also used for inoculation in May and November 1936. By teasing out the perithecia from the bark and suitable manipulation in water considerable numbers of ascospores were obtained free from admixture with conidia. Aqueous suspensions of ascospores were applied to freshly cut extremities of stems of the cultivated varieties or to T-shaped cuts made in the stems. All these inoculations were successful, resulting either in dying back of the stems or in the formation of cankers. Both conidial pustules and perithecia developed in the dead bark, thus providing further evidence of the relationship of the conidial stage to *G. corticola*.

Some inoculation experiments were carried out in October 1934 and October 1936 with Etoile de Hollande and Golden Emblem roses growing in pots in an unheated glasshouse, conidial suspensions of isolations nos. 1–5 being applied to freshly cut stem extremities. Infection resulted, but there was less die-back than with comparable inoculations in the

fields, doubtless owing to the earlier awakening of the host from dormancy in the glasshouse and consequent checking of the parasite. During the 1934 experiments the glasshouse atmosphere was dry and few conidial fructifications developed on the dead bark. During the experiments begun in October 1936 the atmosphere of the glasshouse was kept moist with the result that abundant conidial pustules developed. Ascospore inoculations were also successful in the glasshouse.

<div align="center">PATHOLOGICAL ANATOMY</div>

The cankers caused by this parasite were studied anatomically at different stages of development. The mycelium spreads between the cortical cells, killing them as growth proceeds. The walls and contents of the cells become brown, and after death they may be penetrated by the hyphae. The mycelium may eventually reach the wood by way of the medullary rays, and brown gum is deposited in the cells. Occasionally the pith may be invaded, when it also becomes brown. Sometimes, however, the fungus enters the wood to no great depth, when a narrow darker zone of gummy material delimits the diseased from the healthy wood. In the cankered bark the mycelium spreads more rapidly in a longitudinal than in a lateral direction, and stabilized cankers are often seen which extend only partly round the stem. During summer the healthy bark on the margin of the canker forms a phellogen which produces a cork barrier that extends from the surface to the wood, thus preventing the extension of the canker during the current season. The xylem cells formed by the cambium on the border of the canker remain parenchymatous, and this region may also be traversed by a cork barrier continuous with that in the bark on the margin of the lesion. Opportunity for the lateral extension of the canker by invasion of the newly formed xylem is thus greatly reduced. In our experience further development of the canker after its progress has once been checked in this way is very rare. On the other hand, if the canker spreads all round the stem before it is delimited by a cork barrier, the stem above the canker is completely killed and the disease simulates a die-back.

It has been pointed out that where infection occurs through the cut extremities of stems, as in pruning wounds, extensive die-back may result, unassociated with the formation of cankers. A few words will first be said about the reactions of rose tissues exposed by pruning apart from invasion by a parasitic fungus. Study of these reactions shows that they are very similar to those in plum stems described by Brooks & Brenchley

(1931). Soon after wounding, the woody tissues become brown from the surface downwards owing to the formation of gum in the vessels and medullary rays, with the coincident disappearance of starch. This may be wholly a wound response or it may be associated with invasion by harmless micro-organisms. The browning of the tissues extends downwards in a V-shaped manner until a bud or lateral shoot is reached, at about which level a narrow darker zone of gum is formed. This has been termed a "gum barrier" as it appears to check parasitic invasion. The pith in a rose stem is wider than in a plum stem, and in the former a "gum barrier" is also formed at the appropriate level in the small living cells of this region. Corresponding with the "gum barrier" in the pith and wood a cork barrier is formed in the bark. Apart from parasitic invasion rose "snags" always die in this way. The time rate of these changes depends on the season of the year when the wound is made. If it is made in the winter the host reactions do not occur rapidly until spring, but if the tissues are exposed during spring or early summer the reactions follow speedily and the wound is soon rendered relatively innocuous.

When wounds such as those made in pruning are invaded by the parasite under consideration it grows downwards in the bark and wood, killing the tissues as it proceeds. The progress of the fungus in the wood is accompanied by the formation of gum. The hyphae pass from cell to cell through the pits, but exercise no disintegrating action on the membranes. During the summer, however, the growth of the fungus is usually stayed, after which invasion never appears to be resumed. On sectioning a stem in which invasion has stopped, a narrow, very dark zone of gum is seen at the junction of dead and healthy tissues (wood and pith), which the fungus seems unable to penetrate and which is therefore termed a "gum barrier", similar to that described by Brooks & Moore (1926) in plum stems where invasion by *Stereum purpureum* has been checked. At about the same level in the bark there is a cork barrier which also prevents further invasion. These barriers are usually formed in the vicinity of a lateral shoot. The extent of the die-back caused in this way depends on the time of year when infection begins and on the relative susceptibility of the variety. Evidence was obtained that "gum barriers" are formed more rapidly in resistant than in susceptible varieties of roses.

IDENTIFICATION OF THE PARASITE

The perithecial stage of the fungus has been identified as *Gripho-sphaeria corticola* (Fckl.) v. Höhnel. The genus *Griphosphaeria*, of which *G. corticola* is the type species, was established by von Höhnel (1918) on account of the peculiar character of the perithecial wall, most of which consists of hyphae that run parallel to one another and are closely pressed together. Petrak (1921) also has described *G. corticola* and has stressed the unusual structure of the perithecial wall. Both authors give long lists of synonyms, which include *Metasphaeria corticola* (Fckl.) Sacc. We have compared our fungus with Petrak's material of *Griphosphaeria corticola* on *Rosa canina* in the Kew Herbarium, and we are satisfied that it is the same species. Grove (1937) states that he and Mr E. A. Ellis (of Norwich) repeatedly found *Metasphaeria corticola* on twigs of wild roses in intimate association with *Coryneum microstictum* B. & Br. Mr Ellis has kindly allowed us to examine his specimens of *Metasphaeria corticola* on wild and cultivated roses, which agree exactly with our fungus. Dr G. E. Deacon reported to one of us in April 1935 that he had found perithecia (subsequently determined as *Griphosphaeria corticola*) on stems of rose stocks and cultivated varieties near Norwich. A survey of the literature shows that the fungus now known as *G. corticola* occurs commonly on dead stems of *Rosa*, *Rubus*, *Crataegus*, *Prunus* and other genera in Central Europe. As there is no English description of *Griphosphaeria corticola* the following diagnosis is given:

Perithecia scattered or aggregated, sunk in the substratum, brownish black, globose, $400-600\mu$ in diameter, tapering upwards to the ostiole which becomes visible on the rupture of the overlying tissues; most of the wall consisting of two layers of closely compressed, parallel hyphae, the inner one hyaline and the outer one brown; asci cylindrical, thickened at the apex, $80-120 \times 7-8\mu$, eight-spored; ascospores obliquely uniseriate, spindle-shaped, straight or somewhat one-sided, tapering towards the rounded ends, mostly three-septate, sometimes slightly constricted at the septa, $14-18 \times 5-7\mu$, hyaline; paraphyses numerous, slender, longer than the asci, $2-3\mu$ wide.

Illustrations of the perithecia are shown in Pl. XV, figs. 1, 2.

Greater difficulty has been experienced in naming the conidial stage. It has already been pointed out that some of the conidial isolations have given rise to perithecia whereas others have not. Another complication is that the conidial fructifications range from imperfectly formed

pycnidia (*Hendersonia*) to typical acervuli (*Coryneum*) even as regards the same isolation (Pl. XIV, figs. 3, 4). Such variation in development is in agreement with the conclusion of Archer (1926), concerning an allied fungus, who found that *Hendersonia Rubi* (West.) Sacc. is identical with *Coryneum ruborum* Oud. A further complication arises from the fact that the conidia of the various isolations, although all predominantly three-septate and brown (except for the usually hyaline basal cell), differ somewhat in shape, size and colour; these conidial isolations also show slight differences in cultural behaviour and in relative pathogenicity. Since, however, all these conidial isolations produce the same kind of disease in roses and since they are closely similar in structure, we incline provisionally to the view that they represent a complex of slightly differing types which in the present state of our knowledge should be referred to a single species. This view is fortified by the opinion of Grove (1937) who suggests that *Coryneum (Coryneopsis) microstictum* B. & Br., *Hendersonia canina* Brun., and *H. Henriquesiana* Sacc. & Roum. are all probably identical. Many other species of *Hendersonia* are recorded on roses in Saccardo's *Sylloge Fungorum*, and it is likely that most of these also are synonymous with *Coryneum microstictum*. Material of several of these species of *Hendersonia* and of *Coryneum microstictum* on roses preserved in the Herbaria of Kew and the British Museum (Natural History) has been examined and compared with our own specimens. Some of the specimens labelled as species of *Hendersonia* in these Herbaria are unquestionably identical with *Coryneum microstictum*. With regard to our own conidial collections, some agree exactly with *C. microstictum*, including those associated with perithecia of *Griphosphaeria corticola*; others diverge somewhat from it, but may nevertheless be included in it in view of the remarks made above. It seems best, therefore, to refer all the conidial isolations provisionally to *Coryneum microstictum*, some of which only have formed perithecia during these investigations. It is not unusual for certain strains of other Ascomycetes to lose the capacity of forming ascocarps. On the other hand, further research may show that not all the conidial isolations can be included in *C. microstictum*. Like *Griphosphaeria corticola*, *Coryneum microstictum* occurs on many other hosts as well as on roses. The following description of *C. microstictum* is given in the light of our own studies:

Acervuli or ill-defined pycnidia brownish black, sunk in the sub-stratum and only partly exposed on the rupture of the host tissues; conidiophores slender, unbranched, $20-25 \times 1 \cdot 5\mu$. hyaline; spores pear-shaped, ovoid or spindle-shaped, straight or rarely slightly curved,

mostly four-celled, light to dark brown except for the basal cell which is usually hyaline, $12-18 \times 4-6\cdot5\mu$.

DISCUSSION

Hitherto only slight references have been made to this disease in accounts of the pathology of roses, although it is undoubtedly widely distributed. *Coryneum microstictum* and *Griphosphaeria corticola* have been frequently described from the morphological standpoint on dead stems of roses and other plants without reference to pathogenicity. Sorauer (1888) briefly described two *Hendersonia* diseases of roses which may perhaps be related to the disease described in this paper. Beauverie (1914) described a disease of roses in France which was associated with *Coryneum microstictum*. Averna-Saccá (1926) described a stem disease of cultivated roses in Brazil, which he attributed to *Hendersonia rosicola* n.sp. It appeared in the form of large spots or livid rings and sometimes caused wilting. Mr W. C. Moore (of the Plant Pathological Laboratory, Ministry of Agriculture) tells us that Miss Westcott and Miss Jenkins (from the United States) found a canker of roses at the Royal Horticultural Society's Garden at Wisley in 1930 which was associated both with a *Coryneum* or *Hendersonia* and perithecia of *Griphosphaeria corticola*. In *Bulletin* 79 of the Ministry of Agriculture (*Fungus and other Diseases of Crops*, 1928–1932) mention is made of the occurrence of a species of *Coryneum*, probably *C. microstictum*, on roses in Surrey in 1931 and in Monmouth in 1932. Jenkins (1937) has described a canker of roses in various parts of North America which is caused by *C. microstictum*.

This disease of roses, although fairly common in our experience, rarely occurs abundantly and is therefore liable to be overlooked. From our observations at the Field Station of the Cambridge Botany School, however, this canker and die-back can cause considerable damage to susceptible varieties of roses if allowed to spread without check. It occurs most frequently on certain varieties of hybrid tea roses, and as these are usually drastically pruned in the spring the lesions are generally cut off and destroyed. The destruction of the disease by fire is, in fact, the only course that can be recommended. With the Poulsen varieties of roses, which also are susceptible to the disease, cutting out of the affected parts will have to be done deliberately as these varieties are only pruned lightly if at all. The disease has also been reported on roses grown commercially under glass, but control should be easy under these conditions.

Summary

1. A canker and die-back of roses caused by *Griphosphaeria corticola* (Fckl.) v. Höhnel is described.

2. Conidial fructifications, which may be acervuli or ill-defined pycnidia, precede the formation of perithecia. Conidial isolations are of slightly differing types as regards size and shape of spores, cultural behaviour and relative pathogenicity, but at present they are all referred provisionally to *Coryneum microstictum* B. & Br. Some of the conidial isolations have given rise to perithecia, but others have not.

3. Successful inoculations both with conidia and ascospores are described.

4. In Nature, infection by this parasite may occur through pruning wounds, dead buds, leaf scars, wounds made by thorns, and rust lesions.

5. The following varieties of roses appear to be particularly susceptible to the disease: Duchess of Atholl, Lady Inchiquin and Golden Emblem.

6. The pathological anatomy of the disease is described. Infection may occur at all times of the year except summer, when the rapid formation of "gum barriers" by the host tissues checks invasion.

7. The disease is not likely to become widespread unless neglected. Diseased shoots should be cut out; with some varieties normal pruning operations should sufficiently control the disease.

REFERENCES

Archer, W. A. (1926). Morphological characters of some Sphaeropsidales in culture. *Ann. mycol., Berl.,* 24, 46.

Averna-Saccá, R. (1926). *Contribuição para o estudo das molestias cryptogamicas das Roseiras.* São Paulo. (Abstract in *Rev. appl. Mycol.* 6, 487.)

Beauverie, J. (1914). Étude de deux maladies des rosiers. *Premier Congrès International de Pathologie Comparée.* Paris 1912. *Comptes Rendus,* 2, 968.

Brooks, F. T. & Brenchley, G. H. (1931). Silver-leaf disease. VI. *J. Pomol.* 9, 1.

Brooks, F. T. & Moore, W. C. (1923). On the invasion of woody tissues by wound parasites. *Biol. Rev.* 1, 56.

—— —— (1926). Silver-leaf disease. V. *J. Pomol.* 5, 61.

Deacon, G. E. (1938). Notes on rust of briar. *Rose Annual,* 175. Published by the National Rose Society.

Grove, W. B. (1937). *British Stem- and Leaf-Fungi,* 2, 328–30. Camb. Univ. Press.

A Canker and Die-back of Roses

Höhnel, F. von (1918). Mycologische Fragmente. *Ann. mycol., Berl.,* **16,** 86.

Jenkins, A. E. (1937). *Coryneum microstictum* on rose from Oregon. *Mycologia,* **29,** 725.

Petrak, F. (1921). Mykologische Notizen II. *Ann. mycol., Berl.,* **19,** 32.

Sorauer, P. (1888). In Deutschland beobachtete Krankheitsfälle. *Z. PflKrankh.* **8,** 224.

EXPLANATION OF PLATES XIV AND XV

PLATE XIV

Fig. 1. Young canker on a stem of Kokulensky's rose stock. Infection probably occurred through a rust lesion. × 1.

Fig. 2. Cankers on a stem of a cultivated variety of rose, with conidial pustules. × 1.

Fig. 3. An acervulus-like type of fructification. × 170.

Fig. 4. A pycnidial-like type of fructification. × 170.

PLATE XV

Fig. 1. Camera-lucida drawings of *Griphosphaeria corticola.* (1) Perithecium: *cu,* cuticle, etc.; *co,* cortex; *os,* ostiole; *i,* inner part of wall; *o,* outer part of wall; *as,* ascus; *pa,* paraphysis. (2) Asci: *as,* ascus; *s,* spore; *ia,* immature ascus. (3) Portion of perithecial wall: *i,* inner part; *o,* outer part.

Fig. 2. Photograph of perithecium. × 100.

Fig. 3. Cultures of four different conidial isolations on Dox's agar.

(Received 3 September 1938)

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

BROOKS and ALAILY.— A Canker and Die-back of Roses caused by
Griphosphaeria corticola (pp. 213–226)

Fig. 1.

Fig. 2.

Fig. 3.

BROOKS AND ALAILY.—A CANKER AND DIE-BACK OF ROSES CAUSED BY
GRIPHOSPHAERIA CORTICOLA (pp. 213-226)
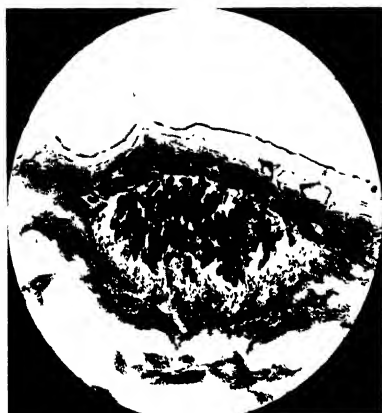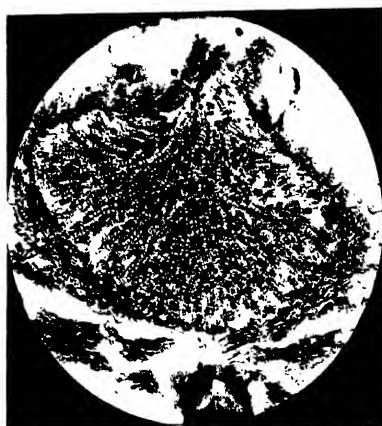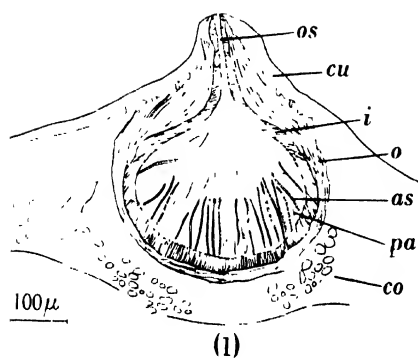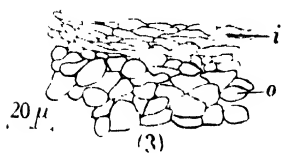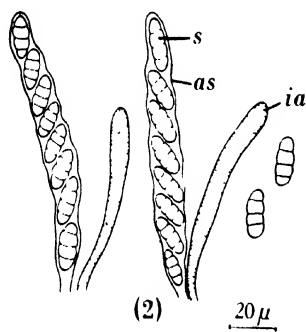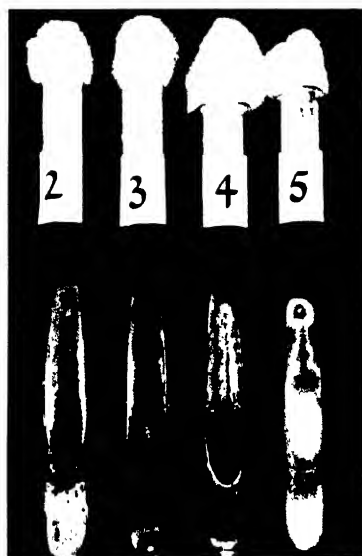
A STUDY OF CERTAIN SPECIES OF THE GENUS *SCLEROTINIA*

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(With Plate XVI)

CONTENTS

INTRODUCTION

WHILE studying *Sclerotinia serica* n.sp. (Keay, 1937) a comparative study was also made of three other species of *Sclerotinia*. Two strains of *S. Sclerotiorum* de Bary were used, one from a diseased swede and one from sclerotia on diseased hop stems. Of five strains of *S. Trifoliorum* Eriks. two were from clover (*Trifolium pratense* L.), the other three from vetch (*Vicia sativa* L.), sainfoin (*Onobrychis viciaefolia* Scop.) and a diseased carrot, the last mentioned being worthy of note since the sclerotinial rot of carrots is generally due to *Sclerotinia Sclerotiorum*. The strains are distinguished by the name of the host following that of the species. Isolations were also made from plants of *Vicia Faba* L. on which sclerotia were conspicuous. These cultures varied somewhat from those of *Sclerotinia Trifoliorum* and were markedly different from those of

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S. Sclerotiorum and *S. serica.* In May 1936 plants of *Vicia Faba*, growing near Newmarket, were seen to be rotted at or below soil level and numerous sclerotia were conspicuous.   Cultures established from mycelium and sclerotia were identical with those of the first isolation, thus substantiating the view that the *Sclerotinia* attacking *Vicia Faba* is distinct from that infecting clover and other leguminous crops.   Cultures established later from sclerotia from diseased beans in Oxfordshire and Wiltshire proved to be identical with those of the two previous isolations. This fungus would seem to be a variety of *Sclerotinia Trifoliorum* and has been given the varietal name *Fabae.* The different isolations are distinguished by roman numerals.

After making several consecutive cultures of *S. Trifoliorum* var. *Fabae* (I) light brown sclerotia, which failed to darken, developed in one tube.   This aberrant form has remained constant although inoculated repeatedly on to lettuce and bean and reisolated.

Isolations from the pith of blackened stems of *Helianthus tuberosus* L., growing in the Cambridge Botanic Gardens, were similar in appearance to *Sclerotinia minor* Jagger.   This appears to be the only record of *S. minor* occurring in Great Britain. The fungus is referred to as *S. minor* (*H. tuberosus*) to distinguish it from a culture of *S. minor* obtained from the Central Bureau voor Schimmelcultures, Baarn.

PATHOGENICITY

(1) *Methods*

To prevent abnormality due to prolonged culturing the fungi were inoculated at frequent intervals on to appropriate hosts and reisolated. Recent "reisolations" were used in all tests for pathogenicity, and the inocula were cut from the edge of actively growing cultures on malt extract or oatmeal agars and placed on an unwounded surface.   The inoculated plants were kept under bell-jars standing in trays of water in a greenhouse. Where the organisms were pathogenic they were reisolated from the diseased tissues.

(2) *Experimental results*

The results of the experiments are summarized in Table I, the maximum infection by each fungus being indicated.   The strain of *Sclerotinia Sclerotiorum* from hop was not so virulent as that from swede, but both infected the same plants with the exception of *Spergula arvensis* L.   More marked differences occurred between the two strains of

Sclerotinia minor, the one from *Helianthus tuberosus* being the more pathogenic. No one strain of *Sclerotinia Trifoliorum* or of the variety *Fabae* was consistently more pathogenic than another. The variant, however, differed considerably for it attacked virulently *Gypsophila elegans* Bieb. and *Cichorium Intybus* L., which were unaffected by the parent strain, and it was more pathogenic than the normal form to *Silene gallica* L. and *S. maritima* With.

Table I. *Results of inoculation experiments*

	Cerastium arvense	Dianthus barbatus	Dianthus deltoides	Gypsophila elegans	Lychnis alba	Silene gallica	Silene maritima	Spergula arvensis	Stellaria media	Cichorium Intybus	Lactuca sativa	Helianthus tuberosus	Onobrychis viciaefolia	Pisum sativum	Trifolium pratense	Vicia Faba	Vicia sativa
S. serica	a	a	b	d	d	d	d	d	d	b	d*	a	.	c*	a	b*	.
S. Sclerotiorum:																	
Swede	b	d	a	d	d	c	d	b	a	d	d	d	d	d	d	d	d
Hop	.	.	.	c	b	b	b	a	.	d	d	d	d	d	c	c	d
S. minor:																	
Baarn	a	.	a	c	a	a	c	a	.	d	d	d	.	d	a	a	.
H. tuberosus	a	.	b	d	c	c	c	c	a	d	d	d	.	d	c	d*	.
S. Trifoliorum var. Fabae:																	
I	.	.	.	a	a	a	c	a	.	.	d	a	b	d	d	d	d
II	.	.	.	a	a	a	b	a	.	a	d	a	b	.	.	d	d
III	.	.	.	a	a	a	b	a	.	a	d	a	a	.	d	d	.
IV	.	.	.	a	a	a	a	a	.	a	d	a	.	.	d	d	.
Variant	.	.	.	d	a	c	d	a	.	d	d	a	a	.	.	d	d
S. Trifoliorum:																	
Vetch	.	.	.	a	a	a	a	a	.	a	d	a	d	d	d	d	d
Sainfoin	.	.	.	b*	a	a	c	a	.	c	d	b	d	d	d	d	d
Clover I	a	a	.	b*	a	a	a	a	.	b	d	a	.	d	d	d	.
Carrot	.	.	.	a	b	b	c	a	.	.	d	a	.	d	d	d	.
Clover II	.	.	.	b*	.	.	.	.	.	.	d	.	.	.	d	d	.

* Young plants.
a = Non-pathogenic.
b = Slightly pathogenic.
c = Considerable rotting.
d = Plants killed.

Little distinction can be made between *Sclerotinia Sclerotiorum* (swede) and *S. minor* (*H. tuberosus*). Although in many instances the latter rotted the plants more rapidly, both species were virulent pathogens. *S. minor* (*H. tuberosus*) was more pathogenic to several members of the Caryophyllaceae than *S. Sclerotiorum* (hop), and on *Spergula arvensis* was more pathogenic than either strain of *Sclerotinia Sclerotiorum*. On some plants *S. minor* (Baarn) behaved similarly to *S. Sclerotiorum* (hop), but on others there were considerable differences. The differences, however, between *S. minor* (Baarn) and both strains of *S. Sclerotiorum*

were no greater than those between the former and *S. minor* (*H. tuberosus*) and it was impossible to separate these two species with regard to their pathogenicity.

Comparing *S. serica* with *S. Sclerotiorum* and *S. minor* the second behaved differently from the other two on *Cerastium arvense* L. and also differed from *Sclerotinia serica* on *Dianthus barbatus* L. *Gypsophila elegans* was severely rotted by all three species, but *Dianthus deltoides* L. was attacked slightly by *Sclerotinia serica* and *S. minor* (*H. tuberosus*) only. Although *S. serica* and the more virulent strain of *S. Sclerotiorum* and *S. minor* all attacked *Lychnis alba* Mill., *Silene gallica* and *S. maritima*, the behaviour of the last two fungi varied in different experiments. *Sclerotinia serica* was markedly pathogenic to *Stellaria media* Cyrill. and *Spergula arvensis*, a common weed on light sandy soil of the type on which the original diseased *Gypsophila* plants were growing. *Sclerotinia serica* can also be distinguished by its behaviour on *Cichorium Intybus*, *Helianthus tuberosus, Pisum sativum* L., *Vicia Faba* and *Lactuca sativa* L. On lettuce the mycelium could only gain an entrance through the young leaves, and beans and peas were not attacked unless they were inoculated before the first foliage leaves had unfolded. It is evident that *Sclerotinia serica* differs in pathogenicity from *S. Sclerotiorum* and *S. minor*, and it is also distinguishable by the silky mycelium produced on diseased tissues and its slower rate of infection.

Excluding the variant of *S. Trifoliorum* var. *Fabae*, the pathogenicity of this fungus and that of *S. Trifoliorum* was the same, a fact of considerable practical importance. They were markedly different from *S. serica*, since they were unable to attack caryophyllaceous plants, but severely rotted the leguminous plants to which *S. serica* was only weakly pathogenic.

Records in the literature state that various plants may be infected naturally by both *S. Sclerotiorum* and *S. Trifoliorum* and Ghamrawy (1932) found no differences in pathogenicity between them. The writer's experiments showed that, by their behaviour on members of the Caryophyllaceae and on *Helianthus tuberosus* and *Cichorium Intybus*, *Sclerotinia Trifoliorum* and the variety *Fabae* were distinguishable from *S. Sclerotiorum* and *S. minor*.

On the basis of this work the fungi were classified into three groups:

    (1) *S. Sclerotiorum* and *S. minor*.

    (2) *S. Trifoliorum* and *S. Trifoliorum* var. *Fabae*.

    (3) *S. serica*.

The value of such data in the delimitation of species is discussed later.

(3) *Infection by ascospores*

Experiments showed that the ascospores of *S. Sclerotiorum* and *S. minor* infect lettuce if they fall on yellowing leaves or on wounds caused by a hot scalpel. Infection was never initiated through a healthy leaf. That healthy tissue is infected by ascospores of *S. Trifoliorum* is suggested by the experiments of Rehm and Coleman, quoted by Wolf & Cromwell (1919). The writer found it impossible to infect clover by spraying it with an aqueous suspension of ascospores of *S. Trifoliorum*. Infection, however, resulted from apothecia developed from sclerotia buried round clover plants. When the apothecia were expanding several of the leaves and stems near ground level were dead or dying. The plants were put under bell-jars and the fungus became established on the moribund tissues and then spread to the healthy portions.

These results support the observations of other investigators that ascospores of *Sclerotinia* spp. are unable to infect healthy cuticularized tissues. In view, however, of the fact that Wilson (1937) has found that spores of *Botrytis cinerea* Pers. can penetrate adult unwounded epidermis, an ability not previously demonstrated, the whole question is in need of further study.

(4) *Infection through the soil*

It has been suggested that sclerotia of *Sclerotinia* may produce mycelium in the soil. Numerous sclerotia were buried in damp sand to obtain apothecia, but the production of mycelium by them was never observed. Since lack of detritus might have prevented such development (see Böning, 1933), whole and half sclerotia were soaked in water for 24 hr. and then buried in the soil around susceptible plants in pots. None of the plants was infected and there were no signs of mycelium having grown out from the sclerotia.

In the writer's opinion sclerotia in the soil rarely, if ever, produce mycelium and cannot be considered a source of infection by functioning in this way. That mycelium of *S. serica* and *S. Sclerotiorum* can cause infection through the soil was shown by an experiment. Mycelial mats from liquid cultures were ground-up with sand and mixed with soil which was placed round plants of *Gypsophila elegans* and *Lactuca sativa*. Both fungi severely rotted the plants and were recovered from the diseased tissues.

MORPHOLOGY

(1) *Production of apothecia*

A marked characteristic of *Sclerotinia serica* is the production of numerous apothecial stipes in cultures aged from 6 weeks to 6 months, especially those on Dox's agar. Development may begin in the dark, but they fail to mature into apothecia unless exposed to light. The majority of the stipes shrivel when a few millimetres long or the tips become swollen, urceolate and covered with microconidia, no asci developing. The production of mature apothecia could not be correlated with the age of the culture nor with the conditions of light and temperature. Apothecial stipes also arose in single-spore cultures two of which produced several mature apothecia. If the microconidia are spermatizing agents the fungus is, therefore, self-fertile. Exposing the other species in tube cultures on several media to various conditions of light and temperature was without result.

The only satisfactory method of obtaining apothecia was the following: small flower pots were partly filled with garden loam and a layer of sand 1 cm. deep; on this the sclerotia were placed, covered with sand, and watered. Large insects were excluded by wire gauze covers and the pots were generally kept outside in trays of water. Unless otherwise stated, sclerotia were not removed from the cultures until microconidia had developed.

(a) *Influence on apothecial production of treatment of sclerotia prior to burial.*

It was hoped to induce the rapid "germination" of sclerotia of *S. Sclerotiorum* and *S. Trifoliorum* by exposing them to different conditions of temperature and moisture before burial. None of the treatments caused more rapid apothecial development than from untreated controls and many sclerotia remained dormant. Analysis of the data suggested no explanation for this. Various investigators have stated that long periods of desiccation do not impair the "germinative" power of sclerotia of these two species. In three instances apothecia of *S. Trifoliorum* developed from sclerotia buried after storage in dry sand for nine weeks. Sclerotia stored in envelopes for 5–14 months did not produce apothecia; the same was true for *S. Sclerotiorum*.

(b) *Influence on apothecial production of the temperature at which sclerotia were formed.*

Sclerotia were buried immediately after removal from cultures employed in experiments on temperature relations. No apothecia developed of *S. serica*, *S. minor* (Baarn) and the variant of *S. Trifoliorum* var. *Fabae*. Sclerotia of *S. Sclerotiorum* formed at 5, 10, 15 and 20° C. produced apothecia. No microconidia had been found in the cultures at 10 and 15° C. when the sclerotia were removed. That no apothecia developed from sclerotia formed at 25° C. agrees with the observation of Kheswalla (1934). Subsequently, numerous sclerotia formed at this temperature were buried without result. Apothecia of *S. Trifoliorum* var. *Fabae* (I) arose from sclerotia formed at 0, 10 and 20° C. and from those of *S. Trifoliorum* formed at 5, 10, 15, 20 and 25° C. In view of the results obtained with *S. Sclerotiorum* it was interesting that sclerotia of *S. Trifoliorum* formed at 25° C. produced apothecia for, whereas the former grew rapidly at 25° C., the latter grew slowly, staled badly and produced smaller sclerotia than at 20° C. Sclerotia of *S. minor* (*H. tuberosus*) formed at 5, 10, 15, 25 and 27° C. produced apothecia. No explanation can be given for the failure to "germinate" of those formed at 20° C. Despite gaps in the evidence it would seem that sclerotia formed over a wide range of temperature can produce apothecia.

(c) *Production of apothecia by single-spore lines.*

No apothecia developed from buried sclerotia from single-spore lines of *S. Trifoliorum* var. *Fabae* and *S. serica*. Sclerotia from ten single-spore lines of *S. minor* (*H. tuberosus*) produced apothecia. Apothecia were also obtained from six single-spore lines of *S. Trifoliorum* (clover I). Seven single-spore lines were begun from one of these apothecia; when the sclerotia were buried four of the lines produced apothecia, thus showing that spores derived from a single-spore line are in no way unusual in their nuclear composition. The majority of sclerotia from single-spore lines of *S. Sclerotiorum* rotted, but three lines produced apothecia, showing that the fungus is homothallic.

(d) *General conclusions on apothecial production.*

General conclusions regarding apothecial production are based on the results of numerous observations during 1934, 1935 and 1936 in addition to those detailed above.

Apothecia of *S. minor* (*H. tuberosus*) were produced from June to December, fewer developing as the weather became colder. The only apothecia which appeared during the first half of the year were in pots in the greenhouse. The sclerotia did not survive more than one year. *S. minor* (Baarn) never produced apothecia.

The sclerotia of *S. serica* frequently decayed rapidly and, although many sclerotia and pieces of plectenchyma were buried, comparatively few apothecia arose. Apothecia developed in April, May, September and October so that extremes of temperature are apparently unfavourable for their development. Twice sclerotia "germinated" very quickly; in April 1935, sixteen apothecia developed from sclerotia and plectenchyma buried 36 days previously and, in May, thirteen apothecia arose from sclerotia and plectenchyma buried 18 days before.

Records in the literature show that the development of apothecia of *S. Trifoliorum* is generally in the autumn, but that it may occur until March if the weather is mild and damp. In the present work apothecia were produced from June to December, the majority appearing in October. Some sclerotia "germinated" a few months after burial, others remained dormant for nearly two years.

Apothecia of *S. Trifoliorum* var. *Fabae* developed at the same time as those of *S. Trifoliorum*, although in 1937 a few of the former developed in February and April. Many sclerotia decayed rapidly, others "germinated" after 15 months' burial. The variant of this fungus never produced apothecia.

Under natural conditions the apothecia of *S. Sclerotiorum* are recorded as developing in spring and early summer. In these experiments no apothecia developed before April. During 1935 apothecia appeared from April till December, the majority in June, and in 1936 from May to November. Sclerotia "germinated" after 30 months' burial.

Temperature and moisture are important factors influencing the "germination" of sclerotia. In these experiments, since the sand was continuously moist, temperature is assumed to be the more important factor. Although apothecial production by *S. Trifoliorum* and the variety *Fabae* showed slightly more connexion with season than that by *S. Sclerotiorum* and *S. minor*, it took place in all four fungi over a wide temperature range. The time of development was independent of the medium on which, or the temperature at which, the sclerotia were formed, or the time at which they were buried.

(2) *Size, shape and colour of apothecia*

The previous nutrition of sclerotia of *S. minor* (*H. tuberosus*) was found to affect the size of the apothecia. Numerous apothecia arose from sclerotia formed on sterilized potato, carrot and artichoke, and 104 had an average diameter of 3·9 mm. and a range of 1·5–9 mm. Seventy-three apothecia, developed from sclerotia from single-spore cultures on malt extract, had an average diameter of 2·2 mm. and a range of 1–5 mm., sixty-two of them being less than 3 mm. in diameter. Twenty-six apothecia of the original isolate grown on the same medium had an average diameter of 1·7 mm. and a range of 1–3 mm., only four apothecia being greater than 2 mm. in diameter. Since the apothecia from sclerotia formed on sterilized vegetables and those from single-spore cultures on malt developed concurrently, the difference in size was apparently due to the conditions under which the sclerotia had formed.

It is unfortunate that *S. minor* (Baarn) did not produce apothecia. Jagger (1920) gives the diameter of the apothecium as 0·5–2 mm., figures which approximate to those for *S. minor* (*H. tuberosus*) from sclerotia produced on malt. One cannot say, however, that the size of the apothecium from sclerotia grown on malt is more "typical" of the strain than that from sclerotia formed on vegetables. It would seem, therefore, that the size of the apothecial disk is of little value for distinguishing these species. This opinion is strengthened when measurements of apothecia of the other species are studied (Table II).

Table II. *Dimensions of the disks of apothecia*

Name of fungus	Average diameter of apothecia in mm.	Range in mm.
S. Trifoliorum	3·9	2 –8·5
S. Trifoliorum var. *Fabae*	2·8	2 –6·5
S. Sclerotiorum	4·3	1·5–8
S. serica	2·5	2 –5

The length of the stalk of the apothecium depends on the depth at which the sclerotium is buried and, consequently, this measurement should not be used as a diagnostic character.

Although the diameter of the disk of the apothecium is valueless for distinguishing these species, the shape and colour of the apothecia are distinctive. Since *S. serica* has already been described (Keay, 1937) it will not be dealt with here. The apothecia of *S. Sclerotiorum* are light brown; they usually develop slightly above the level of the sand and are somewhat trumpet shaped; the central depression is well marked in

young apothecia and is rarely entirely absent in mature disks. Young apothecial stalks of *S. minor* (*H. tuberosus*) are slender and of a uniform colour. In the early stages of development the edges of the tubular tip are inrolled but, as the tip increases in size, the mouth enlarges and the tubular structure becomes more marked; at the same time the stalk darkens and, in the mature apothecium, it is brown while the disk is light buff. As the disk matures it becomes flattened, the centre being slightly raised. Apothecia of *S. Trifoliorum* are distinguishable from those of the other species by their dark colour and tubular shape for, even when mature, there is generally a deep central depression. Frequently the stalk is swollen beneath the disk, gradually increasing in diameter from above the sand level and bearing the cap well above the surface. The apothecia of *S. Trifoliorum* var. *Fabae* are also produced well above the surface and are the same colour as those of *S. Trifoliorum*. The stout stalks are of a uniform width, not being swollen beneath the disks as those of *S. Trifoliorum*, and the disks of the variety *Fabae* tend to be flatter than those of the normal strains. Frequently, however, it was difficult from a cursory examination to distinguish the apothecia of these two fungi.

(3) *Measurements of ascospores and asci*

Measurements of ascospores and asci were made by means of a Leitz Okular Shrauben-mikrometer. A statistical analysis was made of the results, which are presented in Tables III and IV. More than one sample of ascospores and asci of each species was measured. All the measurements of *S. Sclerotiorum* were of the strain from swede. The strain of *S. Trifoliorum* measured was that from clover. Three of the isolations of *S. Trifoliorum* var. *Fabae* were measured. The method of collecting ascospores was to place a sterilized cover-slip, on which was a drop of sterile distilled water, over a discharging apothecium and it is presumed that only mature ascospores were collected. It was more difficult to judge the state of maturity of an ascus, but only those were measured in which the outlines of the eight spores were clearly visible. The values of the means derived from different samples of the same fungus were not identical and the sample with the greatest mean length did not always have the greatest mean breadth. Fisher's "*t*" test (1936) showed that in some instances a significant difference occurred between the mean value for both length and breadth, while in others only one of these values was significantly different. The cause of these variations in spore and ascus size could not be determined. For example, two samples, each

of 100 spores, of *S. Trifoliorum* were measured on the same day from two apothecia which had developed in the same pot from sclerotia formed under apparently identical cultural conditions. The means obtained, $15\cdot09 \times 9\cdot8\mu$ and $14\cdot52 \times 7\cdot62\mu$, are significantly different. On the other hand, the means $18\cdot92 \times 9\cdot34\mu$ and $18\cdot88 \times 10\mu$ for two samples of spores

Table III. *Dimensions of ascospores of four species of* Sclerotinia.
Each sample contained 100 *spores, except* (i) $=50$; (ii) $=35$

Name of fungus	Origin of sclerotia from which apothecia developed	Range μ	Length Mean μ	s.e. μ	s.d. μ	Breadth Mean μ	s.e. μ	s.d. μ
S. Sclerotiorum (swede)	Produced in culture	10–14 by 5–8	11·84	0·08	0·8	6·52	0·05	0·503
	From a rotten carrot (i)	8–15 by 5·5–8	12·44	0·18	1·28	6·63	0·062	0·44
	Produced in culture	10–16 by 4·5–8·5	12·86	0·118	1·177	6·64	0·087	0·875
S. minor (H. tuberosus)	Produced on carrot	10–19 by 6–9	14·1	0·166	1·66	7·87	0·06	0·596
	Produced on malt	10–18·5 by 5·5–10	14·1	0·129	1·295	7·89	0·083	0·827
S. Trifoliorum (clover I)	Produced in culture	8·2–20 by 5–10	14·52	0·216	2·16	7·62	0·11	1·11
	As above. Another apothecium in same pot	11·5–20 by 6·5–13	15·09	0·183	1·83	9·8	0·14	1·4
	Produced in culture	10–18·6 by 4·6–12	15·4	0·19	1·94	8·86	0·158	1·58
	Produced in a single-spore culture	11·5–20 by 6–10	15·9	0·183	1·827	8·4	0·097	0·97
S. Trifoliorum var. Fabae:								
Isolate I	Produced in culture	12–25·2 by 6·4–14	18·7	0·25	2·5	9·15	0·20	2·0
	Produced on malt at 10° C. (ii)	14·2–23·8 by 6·8–14	18·88	0·28	1·68	10·0	0·32	1·88
	Produced on malt at 20° C.	14·4–27·4 by 7–13·7	18·92	0·199	1·99	9·34	0·158	1·58
Isolate II	From diseased beans in nature (i)	10–28·2 by 8–15·2	19·84	0·471	3·33	11·32	0·262	1·856
Isolate IV	Produced in culture (i)	11·6–24·8 by 7·4–13·2	18·76	0·379	2·68	10·04	0·284	1·47
S. serica	Produced in culture	15–26 by 7–13 ·	21·27	0·24	2·43	9·86	0·12	1·22
	Apothecium developed in tube culture on Dox's agar	14–26·6 by 6·8–17·2	20·04	0·282	2·82	10·38	0·179	1·785

of *S. Trifoliorum* var. *Fabae* (I) are not significantly different, although the parent sclerotia had formed in cultures at different temperatures and the time of burial and "germination" was different. The sample of asci of *S. Trifoliorum* with the smaller mean length and breadth arose from a single-spore line, but the ascospores in this apothecium had a greater mean length than those of the original strain. The largest ascospores and asci of *S. Trifoliorum* var. *Fabae* were produced by an apothecium which

arose from sclerotia gathered from diseased beans in nature. In view of the influence of the nutrition of the sclerotia of *S. minor* (*H. tuberosus*) on the size of the apothecia, it is interesting that there was no difference in the size of spores and asci from sclerotia formed on malt extract and sterilized vegetables.

Variation in the size of asci and ascospores of *S. Sclerotiorum* has been described by Ramsey (1925) and Antokolskaya (1932) and the measurements given by Mundkur (1934) and Kheswalla (1934) for two strains in

Table IV. *Dimensions of asci of four species of* Sclerotinia. *Each sample contained* 100 *asci, except* (i) = 75; (ii) = 68; (iii) = 60; (iv) = 93

Name of fungus	Origin of sclerotia from which apothecia developed	Range μ	Length			Breadth		
			Mean μ	S.E. μ	S.D. μ	Mean μ	S.E. μ	S.D. μ
S. Sclerotiorum (swede)	Produced in culture	108–150 by 8·8–14·6	122·6	0·91	9·13	11·7	0·14	1·4
	Produced in culture	108–152 by 6·5–10·5	128·8	1·006	10·06	8·6	0·089	0·89
S. minor (*H. tuberosus*)	Produced on artichoke	128–182 by 9·5–15·4	149·6	1·19	11·93	12·84	0·165	1·65
	Produced on malt	130–180 by 8–14·5	150·1	1·06	10·57	12·0	0·16	1·6
S. Trifoliorum (clover I)	Produced in a single-spore culture (i)	147–191 by 9·3–15·6	165·2	0·95	8·22	12·7	0·152	1·32
	Produced in culture	160–234 by 9·8–16·5	195	1·67	16·66	14·4	0·168	1·68
S. serica	Produced in culture	155–201 by 11–22	178·3	0·97	9·73	16·8	0·227	2·27
	Apothecium developed in tube culture on Dox's agar (ii)	180–250 by 11–21	209·7	1·94	16·0	16·03	0·229	1·896
S. Trifoliorum var. *Fabae*:								
Isolate I	Produced in culture	167–232 by 10–21	199·6	1·35	13·51	16·0	0·209	2·095
	Produced in culture	165–252·5 by 10·75–20·5	200	1·67	16·67	16·29	0·236	2·36
Isolate II	From diseased beans in nature (iii)	195–248·6 by 13·7–20·5	223·4	1·89	14·68	16·96	0·194	1·50
Isolate IV	Produced in culture (iv)	177·4–249·6 by 13·7–22·4	219·2	1·525	14·71	18·23	0·156	1·507

India are considerably different. Considering the data in the literature and the results of the present investigations one has to decide whether such measurements are of any value for distinguishing species. In the writer's opinion such data can be of value, but in describing *S. Sclerotiorum* and *S. Trifoliorum* some authors have given only the range of ascus and ascospore size. The following example shows that this is inadequate. A sample of spores of *S. minor* (*H. tuberosus*) ranged in length from 10 to 18·5μ, one of *S. Trifoliorum* from 10 to 18·6μ; and it

might be thought that the spores had been produced by the same *Sclerotinia*. On analysing the data statistically, however, the means are 14·1 and 15·4μ respectively, and the difference between them is significant.

It may be seen (Table III) that for each fungus the mean values for the different samples of ascospores arrange themselves round a central value. The values in Table V are the averages of the means and show that the fungi differ considerably in their spore size.

Table V. *Average dimensions of ascospores of four species of* Sclerotinia

Name of fungus	Size of ascospores μ
S. Sclerotiorum (swede)	12·38 × 6·59
S. minor (*H. tuberosus*)	14·1 × 7·88
S. Trifoliorum (clover I)	15·23 × 8·17
S. Trifoliorum var. *Fabae* (I, II and IV)	19·02 × 9·97
S. serica	20·65 × 10·12

Moreover, Fisher's "t" test showed that the mean length of the spores of all the fungi differed significantly with the exception of *S. minor* 14·1μ, *S. Trifoliorum* 14·52μ and *S. Trifoliorum* var. *Fabae* 19·84μ and *S. serica* 20·04μ. The values of the means for breadth overlapped so much that the "t" test was not applied.

The data on ascus size in Table IV show that the values of the means for breadth overlap somewhat and that the mean length for *S. serica* overlaps considerably with those for *S. Trifoliorum* and the variety *Fabae*. Moreover, the variation in ascus size between two samples of the same fungus is more marked than that for the ascospores. The values derived by averaging the means for each fungus are given in Table VI, in which the differences between the fungi are more pronounced.

Table VI. *Average dimensions of asci of four species of* Sclerotinia

Name of fungus	Size of asci μ
S. Sclerotiorum (swede)	125·7 × 10·15
S. minor (*H. tuberosus*)	149·8 × 12·42
S. Trifoliorum (clover I)	180·1 × 13·55
S. serica	193·98 × 16·41
S. Trifoliorum var. *Fabae* (I, II and IV)	210·54 × 16·87

On applying the "t" test the means were all found to differ significantly, but this was also true when samples of the same fungus were compared, with the exception of the two sets of asci of *S. minor*.

Although the ascospores and asci of each fungus have a considerable range in size, on the whole, those of any one organism are distinct from those of the other four. It seems justifiable, therefore, to consider that the five organisms in question are distinct.

CULTURAL BEHAVIOUR

(1) *Temperature relations*

A study was made of the influence of temperature upon the rate of the lateral extension of the mycelium of the different organisms on 5 % malt-extract agar in Petri dishes. The minimum, maximum, and optimum temperatures and the time taken by the cultures to reach a size of 6 cm. diameter at 20° C. are given in Table VII. In this work the optimum temperature is that temperature at which the value of the velocity rate of the reaction is highest throughout the whole of the experimental period, i.e. the temperature at which the time for the fungus to reach various diameters is at a minimum throughout the experimental period. The minimum and maximum temperatures are the lowest and highest at which there was any measurable growth.

Table VII. *Temperature relations of four species of* Sclerotinia

Name of fungus	Range °C.	Optimum °C.	Time, in hr., to reach a diameter of 6 cm. at 20° C.
S. Sclerotiorum (1)	0–30	25	40
,, (2)	0–30	20	36·5
S. minor (*H. tuberosus*) (1)	0–30	25	42
,, (2)	0–30	25	36·5
S. minor (Baarn) (1)	5–30	20	47
,, (2)	5–30	20	45
S. Trifoliorum (1)	0–27	20	69
,, (2)	0–27	20	65·5
S. Trifoliorum var. *Fabae**	0–27	20	59·5
,, variant	0–30	25	42
*S. serica**	0–25	20	103

* Average of three experiments.

Column 1 (Table VII) shows that, with the exception of *S. minor* (Baarn), all the cultures made a measurable amount of growth at 0° C. *S. Trifoliorum* and the variety *Fabae*, however, were the only two organisms which continued spreading until the whole of the medium was covered; cultures of the former took 27 days to reach a diameter of 9·1 cm., of the latter 22 days. *S. Sclerotiorum*, *S. minor* (*H. tuberosus*) and the variant of *S. Trifoliorum* var. *Fabae* all ceased to spread at 0° C. after varying periods ranging from 6 to 19 days. In all instances the rate of spread was small, the greatest being by *S. minor* (*H. tuberosus*) which took 10 days to reach a diameter of 1·67 cm. The diameter of cultures of *S. serica* was 4·7 cm. before the mycelium ceased to spread, but the rate of advance was slow as it took the cultures 23 days to reach this size.

S. serica had the lowest maximum temperature, 25° C. That for *S. Trifoliorum* and the variety *Fabae* was 27° C. The variant of the latter fungus, however, resembled *S. Sclerotiorum* and *S. minor* in having a maximum of 30° C. In every instance the rate of spread at the maximum temperature began at a fairly high level and then fell rapidly, and the cultures did not enlarge in diameter after the first three or four days.

The optimum temperature for *S. Sclerotiorum* was different in two experiments. At 25° C. both sets of cultures took 37·5 hr. to reach a diameter of 6 cm.; comparing these values with those in column 3 (Table VII) it will be seen that there was little difference between the rates of spread at 20 and 25° C. The strain of *S. minor* from Baarn had a lower optimum temperature than that from *H. tuberosus*. *S. serica*, *S. Trifoliorum* and *S. Trifoliorum* var. *Fabae* all had an optimum of 20° C. In this feature also the variant of the latter fungus differed from the parent strain as the optimum temperature was 25° C.

The values in column 3 (Table VII) indicate the rate of spread of the mycelium. *S. Sclerotiorum* and *S. minor* (*H. tuberosus*) spread very rapidly; *S. minor* (Baarn) also spread quickly, though consistently slower than the two former fungi. *S. Trifoliorum* and the variety *Fabae* spread more slowly. The variant of the latter differed from the parent strain, equalling in rate *S. Sclerotiorum* and *S. minor* (*H. tuberosus*). *S. serica* was easily distinguishable from the other organisms as the rate of spread was much slower.

Summarizing the results the fungi fall into four groups:

(1) *S. serica.*
(2) *S. Trifoliorum* and *S. Trifoliorum* var. *Fabae.*
(3) *S. minor* (Baarn).
(4) *S. minor* (*H. tuberosus*), *S. Sclerotiorum* and the variant of *S. Trifoliorum* var. *Fabae.*

The fact that the variant which arose from *S. Trifoliorum* var. *Fabae* has such different temperature relations from the parent form might seem to negative the use of such data in the delimitation of species, but this argument is perhaps not legitimate in the present instance. The same conclusion, nevertheless, must be arrived at if one considers the behaviour of the other fungi. *S. Sclerotiorum* and *S. minor* (*H. tuberosus*) are very similar in their relation to temperature although their appearance in culture is quite distinct, their apothecia are of different shape and colour and they produce asci and ascospores, the mean length and breadth of which are different. Also, although the two forms of *S. minor*

could not be separated systematically on morphological grounds, the differences between them with regard to the influence of temperature are greater than those between *S. minor (H. tuberosus), S. Sclerotiorum* and the variant of *S. Trifoliorum* var. *Fabae.*

(2) *Appearance in culture*

The appearance of the fungi in culture on 5% malt-extract agar may be seen in Pl. XVI, figs. 1–6. For a description of the cultural appearance of *S. serica* see Keay (1937). On all the media employed *S. Sclerotiorum* produces more mycelium than *S. Trifoliorum* and the sclerotia of the former are covered by a fine mycelial weft. On oatmeal agar *S. Trifoliorum* stains the medium brown and produces small waxy globules of microconidia; these waxy bodies have never been observed in a culture of *S. Sclerotiorum*. In test-tube cultures on 5% malt-extract agar *S. Trifoliorum* produces haptera which stretch from the edge of the medium to the glass, often having the appearance of a folded fan, with the end flattened against the glass. Frequently microconidia develop on these in great abundance. As well as these haptera a delicate black membrane is often developed on the surface of the glass. Cultures of *S. Sclerotiorum* do not produce these structures.

The form of *S. minor* from *H. tuberosus* produces smaller sclerotia and more fluffy mycelium than the form from Baarn, in which the mycelium is somewhat flocculated. Chivers (1929) found that the sclerotia of *S. minor* decreased in size with decreasing temperature. This was so for the form from *H. tuberosus*, but not for the form from Baarn, in which the size of the sclerotia was little influenced by temperature. On Dox's medium, oatmeal and potato-dextrose agars the cultures exhibited the same differences, the sclerotia of the Baarn form showing a tendency to coalesce. The cultures were indistinguishable, however, on sterilized carrot, potato and artichoke, each producing a continuous sclerotial crust. On all the media used the appearance of these two fungi is quite distinct from that of the other species.

S. Trifoliorum var. *Fabae* resembles the normal form of *S. Trifoliorum* in that the mycelium on all solid media is inconspicuous, and both fungi produce small, waxy globules of microconidia on oatmeal agar. The variety from bean, however, has smaller sclerotia and does not produce the conspicuous haptera which develop in cultures of the strains from clover. Cultures of both fungi grown on liquid carrot extract were indistinguishable.

The variant of *S. Trifoliorum* var. *Fabae* was first noted on account of the pale colour of the sclerotia. The coloration varied according to temperature; at 5° C. they were light cream, at 25° C. light brown. The sclerotia also differ from those of the parent in being larger and smoother, and the cultures produce more aerial mycelium.

Classifying the fungi with regard to their appearance in culture the following groups emerge:

(1) *S. Sclerotiorum* (hop and swede).
(2) *S. minor* (Baarn and *H. tuberosus*).
(3) *S. serica*.
(4) (*a*) *S. Trifoliorum* (clover, vetch, sainfoin, and carrot); (*b*) *S. Trifoliorum* from *Vicia Faba*.
(5) Variant of fungus from *V. Faba*.

DISCUSSION

In deciding upon the systematic position of the fungi studied their pathogenicity has not been considered, as evidence from this source is valueless for differentiating species. The ability of a fungus to parasitize a plant is essentially due to the fact that it can use substances in the host tissue for its own metabolism and two fungi which have entirely different life histories may use the same basic plant substances for their nutrition. Further, it was found possible to modify the pathogenicity of the fungi, for when grown on Dox's agar their pathogenicity was greatly reduced. Evidence, however, derived from both morphological and cultural studies has been taken into account.

A study of the literature revealed no reason for suspecting that the isolates from hop and swede were not *S. Sclerotiorum* and those from clover, carrot, sainfoin, and vetch were not *S. Trifoliorum*. These two fungi have long been considered distinct species and these investigations support this view, for they have demonstrated that the fungi can be distinguished by the structure of their apothecia, the size of their ascospores and asci, and their cultural characteristics.

The morphology and cultural appearance of *S. serica* are different from those of all the other isolates. Since no published description was found which would apply to this isolate it was decided that it was a previously undescribed species. This seemed justifiable since, in the writer's estimate, the degree of difference between this fungus and *S. Sclerotiorum*, *S. minor*, and *S. Trifoliorum* was as great as that between the three latter fungi, which are generally regarded as three distinct species.

The morphology and cultural behaviour of the *Sclerotinia* from *Helianthus tuberosus* are distinct from those of *Sclerotinia Sclerotiorum*, *S. Trifoliorum* and *S. serica*. Although this isolate differed somewhat from a culture of *S. minor* obtained from Baarn, the measurements of asci and ascospores of the former agree well with those made by Jagger (1920). Moreover, Chivers (1929) showed that six strains of this fungus isolated from different plants in widely separated geographic regions displayed differences in their cultural characteristics. There seems no reason then why the fungus from *Helianthus tuberosus* should not be considered a form of *Sclerotinia minor*.

Jagger (1920) was the first to describe the *Sclerotinia* producing small sclerotia as a separate species and it has been accepted as such by many workers. In Cunningham's (1927) view, however, it is a form of *S. Sclerotiorum*, as the differences cannot be "considered as possessing specific value". The writer has shown that the fungus known as *S. minor* is quite distinctive in morphology and cultural characters from *S. Sclerotiorum*. If the view be held that *S. minor* is only a form of *S. Sclerotiorum*, then it would seem to be necessary to regard the two fungi *S. Trifoliorum* and *S. serica* as different forms of the same species.

Considered from a purely morphological point of view it is possible that the isolate from *Vicia Faba* might be regarded as a distinct species. Cultural characteristics, however, should also be taken into account. It is not easy to define a standard whereby the degree of difference between two cultures can be judged, and such comparisons therefore depend on the opinion of the investigator. In comparing cultures of *Sclerotinia Trifoliorum* and the isolate from bean the differences are quite apparent, but it is doubtful whether they are any greater than the differences between the two forms of *S. minor*. The writer considers that it is not justifiable to regard the isolate from bean as a species distinct from *S. Trifoliorum*. Its distinguishing characters are, however, so constant that it is considered to be a variety. The association of the fungus with beans, further evidence of which association has been obtained since the conclusion of the work described above, led to it being given the varietal name of *Fabae*.

SUMMARY

1. Investigations were carried out on *Sclerotinia serica*, two strains of *S. Sclerotiorum* de Bary, five strains of *S. Trifoliorum* Eriks., two forms of *S. minor* Jagger, four isolations of a *Sclerotinia* from *Vicia Faba* considered to be a variety of *Sclerotinia Trifoliorum*, and a variant which arose from one of these isolations.

2. Their pathogenicity to several different plants was tested and the fungi were found to fall into three groups: (1) *S. Sclerotiorum* and *S. minor*, (2) *S. Trifoliorum* and its variety *Fabae*, and (3) *S. serica*.

✗ 3. A variant with light brown sclerotia which arose from *S. Trifoliorum* var. *Fabae* differed from the parent form in pathogenicity.

4. Ascospores of *S. Sclerotiorum*, *S. minor* and *S. Trifoliorum* did not infect healthy leaves.

5. The production of mycelium by sclerotia in the soil was not observed.

✗ 6. *S. serica* differed from the other fungi in that it produced numerous apothecial stipes and several apothecia in test-tube cultures.

7. Exposing sclerotia to different conditions of temperature and moisture after their removal from culture and before their burial in damp sand did not hasten apothecial production.

8. Sclerotia formed in cultures grown over a wide temperature range produced apothecia when subsequently buried in damp sand.

9. Single-spore lines of *S. serica*, *S. Sclerotiorum*, *S. minor* and *S. Trifoliorum* produced apothecia.

10. Apothecial production took place over a wide temperature range.

11. Apothecia of one form of *S. minor* arising from sclerotia formed on sterilized vegetables were larger than those from sclerotia formed on malt-extract. The dimensions of the apothecia are given and a description of the apothecia of *S. Sclerotiorum*, *S. minor*, *S. Trifoliorum* and *S. Trifoliorum* var. *Fabae*.

12. Measurements are given of asci and ascospores.

13. The minimum, maximum and optimum temperatures for linear spread of mycelium are given: also the time taken by cultures to reach a diameter of 6 cm. at 20° C. The view is expressed that such data should not be used for differentiating species. *S. serica* spread more slowly than the other species. The variant of *S. Trifoliorum* var. *Fabae* had different temperature relations from the parent form.

14. A brief description is given of the cultural appearance of *S. Sclerotiorum*, *S. Trifoliorum*, *S. minor*, *S. Trifoliorum* var. *Fabae* and its variant.

15. The classification of the fungi is discussed.

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REFERENCES

ANTOKOLSKAYA, M. P. (1932). The races of *Sclerotinia libertiana* on sunflower and other plants. *Bull. Pl. Prot., Leningr.*, (1), **5**, 39.

BÖNING, KARL (1933). Zur Biologie und Bekämpfung der Sklerotienkrankheit des Tabaks (*Sclerotinia Sclerotiorum* (Lib.) Massee). *Phytopath. Z.* **6**, 113.

CHIVERS, A. H. (1929). A comparative study of *Sclerotinia minor* Jagger and *S. intermedia* Ramsay in culture. *Phytopathology*, **19**, 301.

CUNNINGHAM, G. H. (1927). Fungus diseases attacking artichokes. *N.Z.J. Agric.* **34**, 402.

FISHER, R. A. (1936). *Statistical Methods for Research Workers*. Oliver and Boyd.

GHAMRAWY, ALI K. (1932). A comparative study of *Sclerotinia Sclerotiorum* and *S. Trifoliorum*, with special reference to their physiology and pathology. Thesis for the degree of Doctor of Philosophy, University of London.

JAGGER, IVAN C. (1920). *Sclerotinia minor* n.sp., the cause of a decay of lettuce, celery, and other crops. *J. agric. Res.* **20**, 331.

KEAY, MARGARET A. (1937). An undescribed species of *Sclerotinia*. *J. Bot., Lond.*, **75**, 130.

KHESWALLA, K. F. (1934). *Sclerotinia Sclerotiorum*. Causing stem rot of tobacco. *Indian J. agric. Sci.* **4**, 663.

MUNDKUR, B. B. (1934). *Sclerotinia Sclerotiorum* attacking Patwa. *Indian J. agric. Sci.* **4**, 758.

RAMSEY, G. B. (1925). *Sclerotinia* species causing decay of vegetables under transit and market conditions. *J. agric. Res.* **31**, 597.

WILSON, A. R. (1937). The chocolate spot disease of beans (*Vicia Faba* L.) caused by *Botrytis cinerea* Pers. *Ann. appl. Biol.* **24**, 258.

WOLF, F. A. & CROMWELL, R. O. (1919). Clover-stem rot. *Tech. Bull. N. C. agric. Exp. Sta.* **16**, 18.

EXPLANATION OF PLATE XVI

Fig. 1. Culture of *Sclerotinia minor*, strain from Baarn.

Fig. 2. Culture of *S. serica*.

Fig. 3. Culture of *S. minor*, isolated from *Helianthus tuberosus*.

Fig. 4. Culture of *S. Sclerotiorum*.

Fig. 5. Culture of *S. Trifoliorum* var. *Fabae*.

Fig. 6. Culture of *S. Trifoliorum*.

All cultures grown on 5 % malt-extract agar at 20° C.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.
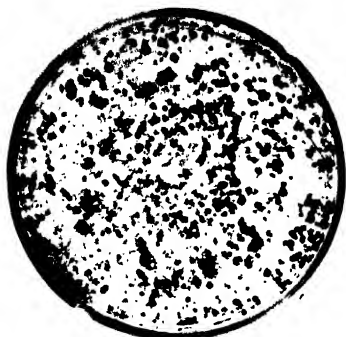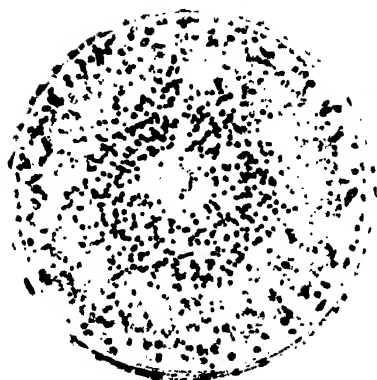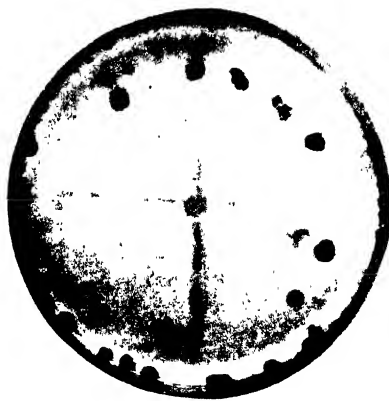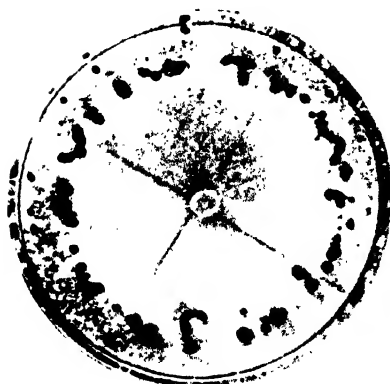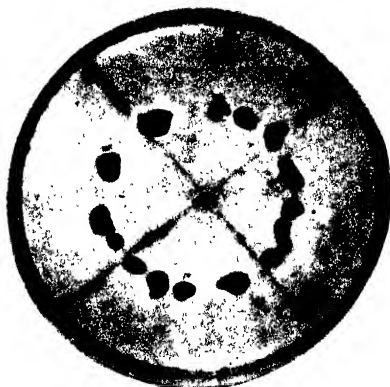
KEAY.—A Study of Certain Species of the Genus *Sclerotinia* (pp. 227–246)

INTERACTION OF SOIL MICRO-ORGANISMS WITH *OPHIOBOLUS GRAMINIS* SACC., THE FUNGUS CAUSING THE TAKE-ALL DISEASE OF WHEAT

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(With 2 Text-figures)

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INTRODUCTORY

THE literature relating to the interaction of micro-organisms in the soil is extensive and has recently been reviewed by Waksman (1937). With regard to the effect of other soil organisms upon *Ophiobolus*, the outstanding contributions are those of Simmonds (1928), Sanford & Broadfoot (1931), Henry (1932), Broadfoot (1933a, 1933b) and Garrett (1934, 1936, 1937, 1938). It has been shown that the pathogenicity of *Ophiobolus* is greater in sterilized than in unsterilized soil and greater in sand than in loam. These effects are explained on the basis of soil factors, in particular of antagonism by micro-organisms. The same antagonism has also been adduced to explain the more or less rapid disappearance of active *Ophiobolus* mycelium from a contaminated soil.

In the present work an attempt has been made to evaluate the part played by particular soil organisms in restricting the parasitic power of *Ophiobolus* and to study the principles concerned. The examination was restricted to those organisms which appear as contaminants in isolations

of *Ophiobolus* from infected roots. If it be true that certain organisms determine the parasitism of *Ophiobolus*, one would expect to find them at one time or another in association with the pathogen.

<center>EXPERIMENTAL</center>

<center>(1) *Soil organisms as determining the recovery of* Ophiobolus
mycelium from the roots of inoculated wheat seedlings</center>

Ophiobolus inoculum makes little or no growth in unsterilized soil. However, under such conditions the inoculum infects the young roots if wheat seed is planted on it, the fungus growing along the roots but as a rule not being recoverable by the plating method after the lapse of some time. The infected roots soon become so rotted by the invasion of secondary organisms that the presence of *Ophiobolus* is hard to establish. It is of interest to determine how long the fungus remains alive in the host tissue itself and what organisms follow. If the soil organisms influence the disintegration of *Ophiobolus* it is those which emerge in association with the pathogen from infected roots at one stage or another that probably take a prominent part in the destruction of the parasite.

A number of representative soil types were used in these experiments. These were as follows:

(1) Richmond Deer Park, pH 4·8—a sandy heath soil.
(2) Jersey St Mary, pH 5·0—a potato soil.
(3) Sand, pH 6·6.
(4) Slough, pH 6·7—a medium loam.
(5) Cambridge, pH 6·8—a black fen soil.
(6) Spalding, pH 6·9—a light loam.
(7) Chelsea Physic Garden, pH 7·2—a black garden loam.
(8) Ramsgate, pH 8·0—a light chalk soil.

Soil samples of the above were adjusted to a suitable water content by Garrett's method (1936), packed into tumblers, seeded with wheat over active inocula of *Ophiobolus* as described by Garrett (1936) and incubated at 20° C. At weekly intervals two sample tumblers were taken from each batch. The soil was removed as far as possible by washing and finally by careful brushing of the roots. The three seminal roots which had grown through the inoculum were secured and the laterals removed. A 1 cm. portion of these roots from immediately below the inoculum was cut off, thoroughly washed in running water for about an hour and sterilized by the silver nitrate method (Davies, 1935). The material was then placed on potato-dextrose agar and incubated at

25° C. At each sampling the number of roots examined varied from ten to twenty-five. The organisms appearing from the roots were examined after about 2 weeks. A record was kept of them and the percentage recovery of *Ophiobolus* noted. Similar samples were taken after 2, 3, 4, 5, 6 and 16 weeks from sowing. The treatment of these was identical with that described.
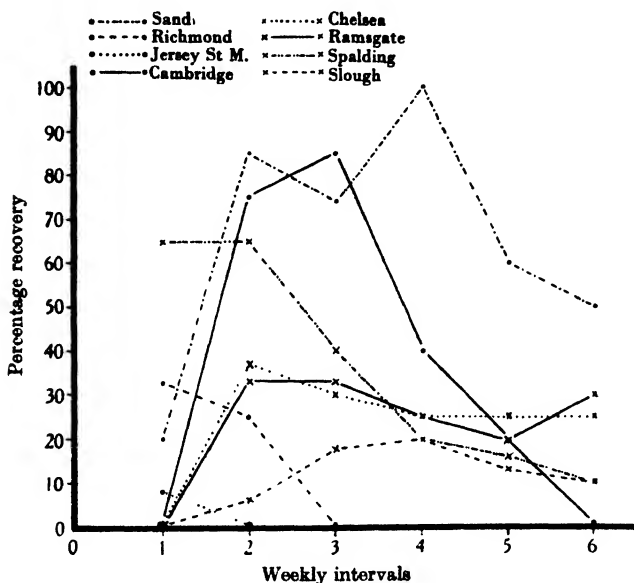
Fig. 1. Percentage frequency of recovery of *Ophiobolus* from infected wheat plants, in various soils at different intervals from inoculation.

The record of one such series of determinations is given in Fig. 1. A repeat experiment gave essentially the same result. In the figure the ordinates represent the percentage frequency of *Ophiobolus* in the various platings, i.e. the percentage of cases in which *Ophiobolus* was recovered in relation to the number of platings.

The general conclusions which may be drawn from the data of Fig. 1 may be stated as follows:

(1) In practically all cases the percentage recovery after 1 week was low. This would readily be interpreted as indicating that the first sampling took place before parasitism of the root was well established.

(2) In all the soils maximum recovery was obtained at about 2–4 weeks from inoculation.

(3) The highest percentage of recovery was obtained from sand, the lowest from acid soils. Also, while the parasite could be isolated freely from infected roots in sand after 6 weeks' attack, it had already disappeared from infected roots in the acid soils by the third week.

(4) Soils of more normal type showed an intermediate behaviour, i.e. the maximum recovery of *Ophiobolus* was lower than in sand but persistence of the parasite was greater than in acid soils. Soil alkalinity apparently favoured the persistence of the parasite.
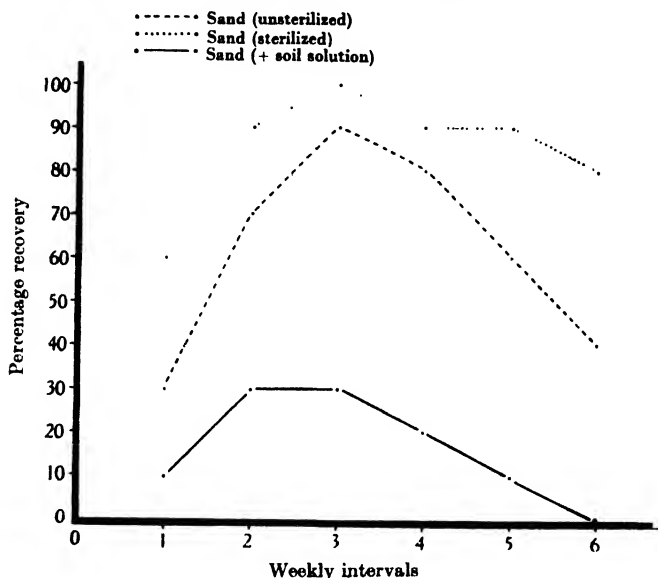
Fig. 2. Percentage frequency of recovery of *Ophiobolus* from infected wheat plants in various modifications of sand culture.

Samplings which were made 16 weeks after inoculation confirmed the above conclusions. *Ophiobolus* was recovered in 20 and 8% of the isolations from sand and Ramsgate (alkaline) soil respectively, but not from any of the others.

The relation of sand as a rooting medium to the persistence of *Ophiobolus* was further tested. A triplicate series of tumblers was set up with sand, (a) unsterilized, (b) sterilized by autoclaving, (c) unsterilized but watered with a soil solution obtained from Jersey St Mary soil. Sowings of wheat over active mycelium of *Ophiobolus* were then made in the manner described above. The results, expressed as previously, are shown in Fig. 2.

Fig. 2 shows that the chance of recovering *Ophiobolus* from infected roots is increased when contaminating organisms are as far as possible excluded and, conversely, that it is lessened when conditions are such as to favour microbial development.

Throughout the experiment of Fig. 1, attention was paid to the identity of the contaminating organisms which appeared in the isolation plates. Complete identification of genus and species was only occasionally possible but, in general, the genus was established. The data obtained are set out in Table I, in which the percentage frequencies of recovery of *Ophiobolus* are also inserted.

Table I. *Contaminants obtained in the isolations of* Ophiobolus *from infected roots of wheat grown in different soils*

Weekly interval	Richmond pH 4·8	Jersey St Mary pH 5·0	Sand pH 6·6	Slough pH 6·7	Cambridge pH 6·9	Spalding pH 6·9	Chelsea pH 7·2	Ramsgate pH 8·0
1	P., Py. (33)	B., F., P.. T. (8)	B., M. (20)	B. (0)	X (0)	B. (65)	F., T. (0)	X (0)
2	F., M., Py., T. (26)	X, M., T. (0)	B. (85)	F., P., T., V. (6)	X (75)	X (65)	Asp. (37)	B., P. (33)
3	T. mainly (0)	Asp., F., R., T. (0)	Asp., P. (64)	T., X (18)	P., T. (80)	T. (40)	B. (30)	Asp., B., P. (33)
4	T., Py. (0)	T. only (0)	X (100)	P., T. (20)	B., P. (40)	Acr., T. (20)	Asp., B., F., T. (25)	P. (25)
5	T. only (0)	X (0)	Asp., B. (60)	Bot., F., T., X (13)	B., Bot. (20)	B., T., X (16)	B., X (25)	B., P., X (20)
6	T. only (0)	T. only (0)	Asp., B. (50)	B., P. (10)	Acr., B., V., X (0)	B., P., T. (10)	B. (26)	B. (30)

Acr. Acrostalagmus; Asp. Aspergillus; B. Bacterium; Bot. Botrytis; F. Fusarium; M. Macrosporium; P. Penicillium; Py. Pythium; R. Rhizoctonia; T. Trichoderma; V. Verticillium; X, unidentified fungi.

Figures in brackets represent the percentage recovery of *Ophiobolus.*

While it is recognized that no clear indication of microbial activity in a soil can be obtained by the plating method, certain tentative suggestions can be made from Table I. In the platings from sand and Ramsgate soil'(in which the persistence of *Ophiobolus* was greatest) *Trichoderma* did not appear, whereas in platings from Richmond soil, which showed minimum persistence of *Ophiobolus*, the dominant isolate after 2 weeks was *Trichoderma*. The remaining soils show an intermediate state, i.e. among the organisms isolated *Trichoderma* was sometimes present and sometimes not. The results thus fall into line with the view which ascribes particular interest to the fungus *Trichoderma* in connexion with the disappearance of *Ophiobolus* mycelium (Weindling, 1932, 1934).

The conclusions to be drawn from the foregoing results may be stated as follows:

(1) The life of *Ophiobolus* in the host tissue is somewhat limited. It persisted longer in sand and in the alkaline soil, being recoverable even after 4 months from the time of inoculation, though the percentage recovery was then much reduced. On the other hand, it disappeared within a few weeks from inoculation in certain acid soils.

(2) Whereas the long persistence of *Ophiobolus* in the sand medium may be ascribed to the comparative absence of soil organisms from the latter, the converse relation applies to soils of the ordinary garden loam type. In particular, it is suggested that when soil conditions favour the development of the fungus *Trichoderma*, they are very unfavourable for the persistence of *Ophiobolus*.

(2) *Soil organisms as affecting the growth of* Ophiobolus *in artificial cultures and in soil*

To ascertain how far the organisms isolated as described above (*vide* Table I) exerted an antagonistic effect upon *Ophiobolus*, a series of growth experiments was carried out on agar plates and in soil.

Cultures on agar plates. Inocula of *Ophiobolus* and of a particular contaminant organism were placed at opposite sides of Petri dishes of standard diameter. The medium used was potato dextrose agar of initial pH 5·8. The plates were incubated at 20° C. At the time when the rate of advance of *Ophiobolus* in the direction of the contaminant was beginning to fall, the width of the clear space separating the two growths was measured. Since the rate of growth of *Ophiobolus* was known from subsidiary experiments to remain fairly constant in the absence of a contaminant, one can ascribe the retardation to a staling effect arising from the contaminant. Further, the greater the distance at which this effect is shown, the greater the antibiotic action of the contaminant concerned. One can thus arrange the contaminating organisms in a series in this respect. Arbitrarily, one can subdivide this series into three groups as follows:

(1) The most strongly antagonistic, which produce an effect on *Ophiobolus* at a distance of 1·5 cm. or more. These are: *Aspergillus* B, *Fusarium culmorum*, *Penicillium* E, H and I, *Rhizoctonia*, *Trichoderma lignorum* B and all bacterial isolates except D, G and H.

(2) Moderately antagonistic, producing an effect on *Ophiobolus* at less than 1·5 cm. and more than 0·5 cm. These are: *Acrostalagmus*

cinnabarinus, Aspergillus A and C, *Fusarium* sp., *Penicillium* C, D, F and G, *Trichoderma lignorum* A, *Verticillium* A and B, and Bacteria D, G and H.

(3) Least antagonistic, producing an effect only at less than 0·5 cm. distance. These are: *Botrytis cinerea, Penicillium* A, B, *Pythium* sp., and two unidentified fungi.

The further behaviour of such double cultures was investigated, viz. as to whether they failed to meet in the long run or whether, if they met, they intermingled or not. In this connexion one can distinguish four types:

A. Cultures not meeting, both organisms becoming staled.

B. Cultures meeting but not intermingling.

C. Cultures meeting, the contaminant growing over the *Ophiobolus* culture and preventing further growth of the latter.

D. Cultures meeting and growing through each other with very little obvious interference the one with the other.

Three isolates, *Fusarium culmorum, Rhizoctonia* sp. and a strain B of *Trichoderma lignorum*, produced a staling effect on *Ophiobolus* at the greatest distance (1·5 cm. or over) and finally completely overgrew the *Ophiobolus* culture and inhibited its growth (group (C) above). One may suggest therefore that, on the medium used, these organisms are the most potent competitors with *Ophiobolus* inasmuch as they check its growth without themselves being materially affected. The least antagonistic effect was shown by a species of *Pythium* which freely intermingled with the *Ophiobolus* without any retarding effect being shown by either.

How far these effects would be shown on other media is not known. A series of experiments with potato-dextrose agar adjusted to initial *p*H 2·8–3·0, 5·8 and 9·2 gave the groupings shown in Table II, the nomenclature being as above. The figures show the number of fungi which fell into each group.

Table II. *Effect of acidity of medium upon degree of antagonism*

Initial *p*H of medium	Group A	Group B	Group C	Group D
2·8–3·0	8	8	6	1
5·8	7	13	3	1
9·2	4	15	3	1

The general suggestion is that acid conditions tend to accentuate staling, e.g. by increasing the number of organisms which act against *Ophiobolus* so as to prevent the cultures from meeting. Acid conditions

also increase the number of organisms which finally grow over the *Ophiobolus* cultures.

Cultures in soil. Twenty grammes of soil were placed over filter paper in Petri dishes containing the requisite amount of water to make up the saturation to 70%. The plates were sterilized at 10 lb. for 1 hr. on three successive days and 5 c.c. of a bacterial or a fungal spore suspension sprinkled uniformly over three plates. These were then inoculated with *Ophiobolus* disks in the centre. The control plates were sprinkled with the corresponding amount of sterile water.

The following results were obtained after 10 days' incubation at 25° C.:

(1) Growth of *Ophiobolus* alone   ...   .   3·56 cm.

(2) Growth of *Ophiobolus* in the presence of *Aspergillus* A and B, *Botrytis cinerea, Fusarium culmorum, Penicillium* A, B, D, E, F, H and I, *Trichoderma* A and B, *Verticillium* A and B   ...   Nil

(3) Growth of *Ophiobolus* in the presence of *Aspergillus* C, *Fusarium* sp., *Penicillium* C and G, *Rhizoctonia* and *Trichoderma* C   ...   .   0·1–0·3 cm.

(4) Growth in the presence of two unidentified fungi   1·0–1·3 cm.

With the *Pythium* sp., which in preceding experiments showed no antagonistic action to *Ophiobolus*, no result was obtained, since the contaminant produced a continuous film over the soil surface, thereby completely obscuring the *Ophiobolus*.

The effect of the bacterial contaminants was in general less marked. Only one of these completely suppressed growth, with several (B, E, F and G) the growth of *Ophiobolus* was reduced to less than 0·5 cm., while the remainder allowed growth exceeding 1 cm.

These two methods of assessing relative antagonistic effect, one on agar plates and the other in soil, show fairly good agreement. On the whole the effects are more pronounced in the soil cultures, as would be expected from the method of inoculation adopted. It is noteworthy that nearly all the contaminant organisms were strongly repressive of *Ophiobolus* growth when inoculated into sterilized soil.

(3) *Metabolic products of soil organisms in artificial culture as affecting the growth and pathogenicity of* Ophiobolus

The effect of soil organisms on the activity of *Ophiobolus* mycelium may be ascribed either to competition for food materials, to the production of substances inimical or even toxic to the latter fungus, or to a combination of both. With a view to separating these factors, the

various contaminant fungi were cultured separately and the medium in which they had grown was tested, either as such or after modification, with regard to its effect on the growth and pathogenicity of *Ophiobolus* mycelium.

As synthetic media are not suitable for the growth of *Ophiobolus*, potato-dextrose solution with 1% dextrose was used. Flasks containing 50 c.c. of this solution were inoculated with a spore suspension of the various contaminants and incubated at 25° C. After 5 days and 21 days sample flasks were withdrawn and the fungus in each case removed by filtration through a Chamberland filter. These media were then used as follows:

A. Undiluted.

B. Diluted to one-half with distilled water.

C. Diluted to one-fourth with distilled water.

D. Diluted to two-thirds with potato-dextrose solution.

E. Undiluted but boiled for 15–20 min.

The various preparations were placed, together with controls of the original unaltered medium, in specimen tubes and each inoculated with a standard disk of *Ophiobolus* mycelium. After 5–7 days, growth was measured. The growth in the controls varied from 2·0 to 2·3 cm., and that in the various used media was as shown in Table III. The second column of the table gives the *p*H reaction of the unmodified used solution A.

The effects are thus somewhat various. Where inhibition of *Ophiobolus* is complete (group I of Table III), none of the treatments of the stale medium (cols. B, C, D, E) has, except in a few cases, caused any improvement in growth. That addition of food (col. D) does not as a rule permit of growth indicates the presence of growth-inhibiting substances. In group III of Table III, the staling effect is not shown, and none of the treatments has produced any material effect. In group II the condition is intermediate. In a few instances (e.g. *Aspergillus* A) high acidity may be the repressing factor, but there is no general rule. When 21-day-old solutions were similarly tested similar effects were shown, but the degree of inhibition was in general less pronounced.

Media staled by the various bacterial isolates gave results of the same kind as those obtained with the fungi, viz. the growth of *Ophiobolus* was either completely inhibited, retarded or unaffected. Addition of food or dilution made little difference. Boiling removed the repressive effect in one case only. The data again suggested the production of substances inhibitory to the growth of *Ophiobolus*.

Table III.   *Effect of fungal metabolic products upon growth of* Ophiobolus

Growth of *Ophiobolus* in cm.

Organisms	pH	A	B	C	D	E
		Group I.	Growth nil			
Aspergillus A	2·8 or less	0·0	0·0	0·0	0·0	0·0
Bot. cinerea	6·4	0·0	0·0	0·0	0·0	Trace
Penicillium C	4·4	0·0	0·0	0·0	0·0	0·0
,, D	4·5	0·0	0·0	0·0	1·2	0·0
,, E	4·6	0·0	0·0	0·0	0·0	0·0
,, F	5·6	0·0	0·0	0·0	0·0	0·0
,, H	5·8	0·0	0·0	0·0	0·0	0·0
Trichoderma A	5·2	0·0	0·0	1·4	0·0	0·0
,, B	--	0·0	0·0	0·0	0·0	2·3
		Group II.	Growth less than 1 cm.			
Penicillium I	7·3	0·9	0·0	1·0	2·0	1·6
Verticillium A	5·8	0·5	Trace	1·7	2·1	0·0
		Group III.	Growth over 1 cm.			
Acrostalagmus cinnabarinus	7·2	1·9	2·1	2·0	2·1	1·8
Aspergillus B	5·2	2·3	2·3	2·3	2·3	2·3
,, C	5·7	2·3	2·3	2·3	2·3	2·3
Fusarium A	7·3	2·3	2·3	2·3	1·7	2·3
,, B	7·4	2·0	2·2	2·0	2·2	2·2
Penicillium A	7·6	2·0	1·9	2·0	2·1	2·1
,, G	6·4	1·7	2·1	1·3	2·0	0·0
Pythium	4·8	2·1	2·3	2·0	2·2	1·4
Rhizoctonia	6·4	2·3	2·3	2·3	2·3	2·3
Trichoderma C	5·6	2·0	1·9	2·1	2·3	2·3
Verticillium B	5·2	1·5	1·5	0·8	2·3	1·5

For pathogenicity tests *Ophiobolus* disks were placed for 5–7 days in the 5-day and 21-day-old filtered stale media and presoaked seeds were planted over them in tumblers containing moist sand. The seed and inocula after planting were also covered with sand. The tumblers were incubated at 20° C. for 10–12 days, after which the seedlings were washed and growth of *Ophiobolus* measured by Garrett's method (1936). Illustrative results are given in Table IV.

It is seen that *Ophiobolus* mycelium subjected for 5–7 days to contact with the staled media of certain fungi is rendered non-pathogenic. Similar preparations from other fungi have no effect, and those from yet others show intermediate behaviour. In a few cases (*Penicillium* D. E and G in Table IV) treatment of the *Ophiobolus* inoculum with the fungal preparations increased its pathogenicity.

Metabolic products of bacterial isolates were similarly tested after 15–20 days' growth: infection was suppressed in some cases, retarded in others.

Table IV. *The effect of preparations from 5-day-old cultures of various fungi upon pathogenicity of* Ophiobolus

Organisms	Growth of *Ophiobolus* along the root in cm.		Col. I ÷ col. II %
	Col. I, staled media	Col. II, control	
Group I, showing no attack			
Aspergillus A	0·0	3·5	0·0
Penicillium C	0·0	2·3	0·0
,, F	0·0	2·2	0·0
,, H	0·0	2·8	0·0
Pythium	0·0	2·8	0·0
Trichoderma A	0·0	3·2	0·0
,, B	0·0	3·2	0·0
Group II, showing attack of less than 1 cm.			
Aspergillus C	0·9	2·8	31
Botrytis cinerea	0·7	2·5	28
Fusarium B	0·8	2·8	28
Penicillium I	0·9	2·8	30
Rhizoctonia	0·6	2·8	16
Unidentified A	0·9	2·8	32
,, B	0·9	2·8	31
Group III, showing an attack of 1 cm. or over			
Acrostalagmus cinnabarinus	1·3	2·5	52
Aspergillus B	2·0	2·3	87
Fusarium A	1·5	2·3	65
Penicillium A	2·0	2·5	80
,, D	2·9	2·2	131
., E	3·3	2·2	148
.. G	2·9	2·2	127
Trichoderma C	1·8	3·2	56
Verticillium A	1·2	2·5	48
.. B	1·8	2·3	78

(4) *Soil organisms as affecting the pathogenicity of* Ophiobolus *in soil*

That soil organisms exercise a suppressive effect on *Ophiobolus* was shown by the following preliminary experiment. Presoaked wheat seeds were sown over *Ophiobolus* inoculum in sand which had been used repeatedly for growing wheat and oat seedlings. The sand used for control was sterilized by heat. The tumblers were incubated at 20° C. for 10 days. Examination of the seedling roots after washing showed that while the fungus had advanced 3·5 cm. along the roots in the sterilized sand there was hardly any initiation of attack on the roots of seedlings grown in oft-used sand. This suggests that the antibiotic activity of micro-organisms in the used sand is responsible for the effect.

A detailed experiment was set up to determine the inhibitive influence of individual organisms on the infective capacity of *Ophiobolus* in soil. The organisms employed were those used in the previous sections.

Ophiobolus and other organisms were developed on Slough soil adjusted to pH 7·0, containing 5% of cornmeal in Petri dishes, previous test having shown that such an inoculum of *Ophiobolus* is actively parasitic. Soil with 75% moisture, previously sterilized for three successive days at 10 lb. pressure for an hour, was distributed in sterile tumblers, roughly 200 g. being placed in each. The soil in each tumbler was gently pressed and over this was placed 5 g. of one of the following:

A. Sterilized soil and cornmeal mixture without any organisms.

B. Soil cornmeal mixture inoculated with *Ophiobolus* alone.

C. Equal quantities of *Ophiobolus* inoculum as in B and of the corresponding inoculum of a soil organism.

D. Soil cornmeal mixture inoculated with a soil organism.

Presoaked seeds of wheat treated with 1 : 320 formalin solution for ½ hr. were sown, five in each tumbler. The seeds and the inoculum were covered with sterilized soil, each treatment being replicated as a rule three times. The tumblers were incubated for the first 5 days at 20° C., after which they were removed to the open to allow of the normal growth of the emerging seedlings. Sterile water was sprinkled over the surface of the soil in the tumblers when necessary.

After the lapse of 2 weeks the heights of the plants were recorded, the roots then removed from the soil and washed and the percentage of primary roots discoloured by fungal attack determined. The percentage emergence of seedlings was noted. This varied from 75 to 93 % of seed sown, but as the variation showed no relation to the amount of root attack the figures are omitted. Illustrative data are given in Table V.

A similar range of results was given in experiments with the various bacterial isolates.

The following points may be noted in Table V:

(1) The height of the plants, when *Ophiobolus* inoculum alone is used, is considerably lower (2·7 in.) than in the uninoculated controls (6·2 in.).

(2) The height of the plants in presence of mixed inocula, although very variable (3·7–6·8 in.), is markedly greater than when *Ophiobolus* inoculum alone is used (2·7 in.).

(3) The height of the plants in presence of mixed *Ophiobolus* and contaminant inoculum is, in general, less than in presence of contaminant alone. This difference is most distinct the higher the percentage of root infection (Group III). Marked exceptions are seen in the case of *Penicillium* F and *Fusarium culmorum*. The latter is certainly a root parasite and the same is possible of the former.

Table V. *Effect of soil organisms upon pathogenicity of* Ophiobolus

Type of inoculum	Av. height (in.) of plants in presence of		% infection of primary roots
	Mixed inoculum	Single inoculum	
No organism	—	6·2	0
Ophiobolus alone	—	2·7	100
Fungal group I (infection <10%)			
Ophiobolus			
+ *Fusarium* A	6·4	6·8	0
+ *Penicillium* C	6·0	6·3	9
+ *Pythium*	5·9	6·1	8
+ *Trichoderma* A	6·2	5·8	3
+ *Trichoderma* B	6·8	7·0	0
+ *Verticillium* B	6·3	6·8	5
Fungal group II (infection 10–30%)			
Ophiobolus			
+ *Aspergillus* A	5·8	5·6	20
+ *Aspergillus* C	5·9	6·5	20
+ *Penicillium* B	5·6	6·5	27
+ *Penicillium* E	6·0	6·8	18
+ *Penicillium* F	6·6	3·9	30
+ *Penicillium* G	6·0	6·6	13
+ *Penicillium* I	6·1	6·4	15
+ *Rhizoctonia*	5·8	6·4	24
Fungal group III (infection >30%)			
Ophiobolus			
+ *Acrostalagmus*	4·4	7·0	90
+ *Aspergillus* B	5·3	6·4	63
+ *Bot. cinerea*	3·7	5·8	100
+ *Fus. culmorum*	5·8	4·4*	50
+ *Penicillium* A	4·7	6·8	100
+ *Penicillium* D	4·6	5·8	81
+ *Penicillium* H	4·6	6·8	55

* Some browning of root.

(4) Certain organisms, viz. *Penicillium* F and H, which completely repressed *Ophiobolus* by their metabolic products in artificial medium (see Table IV), are not so effective in suppressing the activity of *Ophiobolus* in soil.

(5) The antibiotic effect of the various organisms on *Ophiobolus* is variable, ranging from nil to complete inhibition of its attack.

SUMMARY

1. Isolations of *Ophiobolus* from infected wheat roots gave the maximum percentage of recovery 2–4 weeks after inoculation. Later the percentage of recovery declined. Disappearance of *Ophiobolus* mycelium was most rapid in acid soils, and slower in sand and in alkaline soil. In soils of more normal type an intermediate behaviour was shown.

2. From such infected roots a large number of soil fungi and bacteria were isolated and, in part, identified. It is suggested that disappearance of *Ophiobolus* mycelium from invaded roots is conditioned by the activity of these contaminants, and more particularly of such species as *Trichoderma lignorum*. Long persistence of the parasitic mycelium in roots growing in sand suggests that this is a poor medium for the growth of micro-organisms.

3. A systematic study in plate cultures of the interaction between *Ophiobolus* and the various soil contaminants isolated showed various degrees of interference with the growth of the former. These effects are described and tabulated. Similar interference effects were seen when *Ophiobolus* was grown in sterilized soil which had been inoculated with each of the contaminants.

4. These effects of the living contaminants can, to some extent, be reproduced by their metabolic products, viz. by filtered cultural solutions in which these organisms have grown. The inhibiting or retarding effects so produced are not due to the abstraction of food substances but to the presence of deleterious metabolites. In certain cases it was shown that *Ophiobolus* mycelium when exposed to such metabolic products was killed. This result was established both by cultural and pathogenic tests.

5. Tests with wheat seedlings planted over *Ophiobolus* mycelium, with or without the simultaneous presence of the various contaminating organisms, showed that the antibiotic effect varied from no appreciable effect to complete inhibition of attack. The various organisms are divided into three groups on this basis.

REFERENCES

BROADFOOT, W. C. (1933 *a*). Studies on the foot- and root-rot of wheat. I. Effect of age of wheat plant upon the development of foot- and root-rot. *Canad. J. Res.* **8**, 483.

—— (1933 *b*). Studies on the foot- and root-rot of wheat. II. Cultural relation on solid media of certain micro-organisms in association with *Ophiobolus graminis*. *Canad. J. Res.* **8**, 545.

DAVIES, F. R. (1935). Superiority of silver nitrate over mercuric chloride for surface sterilization in the isolation of *Ophiobolus graminis* Sacc. *Canad. J. Res.* **13**, 168.

GARRETT, S. D. (1934). Factors affecting the severity of take-all. *J. Dep. Agric. S. Aust.* **37**, 664.

—— (1936). Soil conditions and the take-all disease of wheat. *Ann. appl. Biol.* **23**, 667.

—— (1937). Soil conditions and the take-all disease of wheat. II. The relation between soil reaction and soil aeration. *Ann. appl. Biol.* **24**, 747.

—— (1938). Soil conditions and the root-infecting fungi. *Biol. Rev.* **13**, 159.

HENRY, A. W. (1932). The influence of soil temperature and soil sterilization on the reaction of wheat seedlings to *Ophiobolus graminis*. *Canad. J. Res.* **7**, 198.

SANFORD, G. B. & BROADFOOT, W. C. (1931). Studies on the effects of other soil inhabiting micro-organisms on the virulence of *Ophiobolus graminis* Sacc. *Sci. Agric.* **11**, 512.

SIMMONDS, P. M. (1928). *Rep. Dominion Botanist (Canada) for* 1927, p. 98.

WAKSMAN, S. A. (1937). Associative and antagonistic effects of micro-organisms. I. Historical review of antagonistic relationships. *Soil Sci.* **43**, 51.

WEINDLING, R. (1932). *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology*, **22**, 837.

—— (1934). Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia Solani* and other soil fungi. *Phytopathology*, **24**, 1153.

STUDIES IN BACTERIOSIS. XXIV

STUDIES ON A BACTERIUM ASSOCIATED WITH LEAFY GALLS, FASCIATIONS AND "CAULIFLOWER" DISEASE OF VARIOUS PLANTS. PART III. FURTHER ISOLATIONS, INOCULATION EXPERIMENTS AND CULTURAL STUDIES[1]

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(With Plates XVII and XVIII)

CONTENTS

INTRODUCTION

IN Part I of this series (Lacey, 1936 *a*) it was stated that Tilford, at the 26th and 27th Annual Meetings of the American Phytopathological Society, had reported the isolation of a pathogenic organism from fasciated sweet peas. Tilford (1936) proposed the name *Phytomonas fascians* for this organism, which, according to the description of its cultural characteristics, is identical with the species isolated by the writer from fasciations and leafy galls on various plants. As, however, by a resolution of the 2nd International Congress for Microbiology, 1936, the generic name "*Phytomonas*" for plant pathogenic bacteria is invalid, in the present paper the organism is referred to as *Bacterium fascians* (Migula's system). In addition to sweet peas, Tilford isolated *Bact. fascians* from growths at the base of chrysanthemums and a geranium and, by inoculation, produced fasciations on sweet peas, garden peas, petunia, tobacco, *Gypsophila paniculata* and geranium.

THE ISOLATION OF *BACT. FASCIANS* FROM ABNORMAL GROWTHS
ON VARIOUS HOST PLANTS

Part I of this series (Lacey, 1936 *a*) described the isolation of a pathogenic bacterium from fasciated sweet peas, leafy galls on chrysanthemums, carnations and *Schizanthus* and from "cauliflower" strawberries, and in Part II (Lacey, 1936 *b*) it was stated that isolations of the same organism had been made from galls on *Nicotiana glutinosa*, *Asparagus sprengeri*, *Heuchera sanguinea* and a gladiolus corm. Further details of these and of subsequent isolations are given below.

New isolations of *Bact. fascians* have been made from sweet pea, *Schizanthus*, carnation and chrysanthemum leafy galls, including two varieties of the Cascade chrysanthemum and a witches' broom type of growth on carnation.

The *Nicotiana glutinosa* plant from which the first isolation was made had a large gall surrounding the base of the stem similar in appearance to a chrysanthemum leafy gall. This plant was sent to the writer in March 1936 by Dr G. C. Ainsworth of the Experimental and Research Station, Cheshunt. In March 1937, Dr Ainsworth sent further specimens of *N. glutinosa* bearing very large leafy galls and also one plant of *N. tabacum* with a small leafy gall at the base of the stem. From both of these species typical cultures of *Bact. fascians* were isolated. Dr Ainsworth gives the following information:

"In connexion with the virus disease investigations here we raise several thousand *N. glutinosa* and *N. tabacum* each year and every year a few *N. glutinosa* and a smaller number of *N. tabacum* develop leafy galls at soil level. It has been noticed that the incidence of this disease of tobaccos is frequently correlated with the use of composts prepared from soil in which chrysanthemums (known to be slightly infected with "leafy gall") have been grown, although the soil is all sterilized by baking before use. Not one of the many thousand tomato plants grown at the same time and in the same composts as the tobaccos has ever developed a gall."

Asparagus sprengeri. In February 1936 Miss Walker of Swanley Horticultural College, Kent, sent fronds of *Asparagus sprengeri* bearing nodal galls on the aerial shoots which were similar in appearance to the type of stem gall produced by *Pseudomonas tumefaciens* (Pl. XVII, fig. 1). Some years previously the writer tried to isolate *Ps. tumefaciens* from similar galls on *Asparagus sprengeri* without success; *Bact. fascians* was easily isolated from these new specimens. Later, Miss Walker sent

two more plants of *Asparagus sprengeri* which, in addition to a few small aerial galls, had large masses of growth surrounding the base of the stems, partly above and partly below soil level. These basal galls were of considerably more compact and solid structure than the leafy galls of chrysanthemums, etc., but again numerous virulent colonies of *Bact. fascians* developed on all the isolation plates made from the growths. In June 1937 Dr Pape, of the Biological Station, Kiel, Germany, sent a basal stem gall of *Asparagus sprengeri*, similar to the Swanley specimens, from which a virulent culture of *Bact. fascians*, identical with the English strains, was isolated.

Brown & Weiss (1937) in America reported that they had failed to isolate *Pseudomonas tumefaciens* from fasciated galls on two specimens of *Asparagus sprengeri*, although the neoplasms were believed to be of the crown-gall type. It is probable that in this case, also, the causal agent was *Bact. fascians*.

"Cauliflower" of Heuchera sanguinea. These plants were sent to Dr T. Goodey, of the Institute of Agricultural Parasitology, by a grower who suspected eelworms as the cause of the disease. Dr Goodey found that, as in the case of "cauliflower" of strawberries, a species of eelworm, *Aphelenchoides fragariae* or *A. pseudolesistus*, was living ectoparasitically among the compact bases of the leaves, but he did not consider this to be the primary cause of the trouble and sent the plants to the writer, who isolated *Bact. fascians* from the growths. Dr Goodey sent further specimens of the same trouble a year later, and again numerous colonies of *Bact. fascians* were obtained on all the isolation plates.

The original gladiolus corm was sent by Mr Green, mycologist of the Royal Horticultural Society's Gardens, Wisley, and a second specimen was received later from Dr Ware of Wye Agricultural College. In both these specimens gall tissue had developed in place of the young cormels.

New host plants

Strains of *Bact. fascians* have been isolated from typical leafy galls on dahlia, pelargonium, petunia, crassula, hollyhock, nasturtium (Pl. XVII, fig. 2) and sweet William, and from abnormal growths on the following plants:

Shasta daisy (Chrysanthemum maximum). A group of Shasta daisy plants in a private garden had large leafy galls surrounding the bases of the stems and also fasciated growths, of a witches' broom type, in the axils of many of the lower leaves.

Cardamine triloba. This specimen bore numerous very short fasciated shoots at ground level. Prof. Weiss, the sender, stated that he had seen similar growths in former years on *Primula juliae* in the same garden.

Lilium regale. This plant had two normal buds below ground, but at the bottom node a gall had developed in place of a new bulb. The growth, which consisted of a solid mass of tissue resembling a corm in consistency and appearance, was loosely attached to the stem by a slender stalk.

Forsythia suspensa. Hard, brown, wart-like galls had developed at the nodes of young twigs of this plant in place of the axillary buds (Pl. XVII, fig. 3). Mr Fox Wilson, entomologist of the Royal Horticultural Society's Gardens, Wisley, who had previously examined this specimen, found that the galls were not of insect origin.

Phaseolus multiflorus. A runner bean, sent by Dr H. W. Miles, of Manchester University, had galls similar to those on *Forsythia suspensa* formed high up the stem in the bud portions.

Cheiranthus allionii *and larkspur.* The growths on these two specimens were of the witches' broom type, the main stems being of normal growth but with tufts of very finely divided leafy structures in the axils of the leaves in place of lateral shoots.

Finally, in addition to the asparagus gall mentioned previously, isolations of *Bact. fascians* have been made from leafy galls on pelargonium, petunia, *Aster frikarti, Verbascum densiflorum, Chrysanthemum maximum* and from an extremely fasciated basal shoot of *Chrysanthemum indicum* (Pl. XVII, fig. 4) sent by Dr Pape from Germany. These German strains of *Bact. fascians* were identical with the English strains.

The host range of plants affected by *Bact. fascians* is evidently very wide. Up to the present isolations of this organism have been made from growths on plant species belonging to sixteen different families, as follows: Monocotyledons-Liliaceae, *Asparagus sprengeri* and *Lilium regale*; Iridaceae, gladiolus. Dicotyledons-Caryophyllaceae, carnation and sweet William; Compositae, chrysanthemum sp., aster and dahlia; Crassulaceae, *Crassula*; Cruciferae, *Cardamine trifolia* and *Cheiranthus allionii*; Geraniaceae, pelargonium; Leguminoseae, sweet pea and *Phaseolus multiflorus*; Malvaceae, hollyhock; Oleaceae, *Forsythia*; Ranunculaceae, larkspur; Rosaceae, strawberry; Saxifragaceae, *Heuchera*; Scrophulariaceae, *Verbascum*; Solanaceae, *Nicotiana* sp., *Schizanthus* and petunia and Tropaeolaceae, nasturtium. In view of this wide distribution it is noteworthy that within the families, genera, species and even varieties differ widely in their susceptibility to attack. For example,

among the Solanaceae, petunias are very susceptible to "leafy gall" but tomatoes and potatoes appear to be immune. *Schizanthus retusus* is much more susceptible than other *Schizanthus* species. In the nursery from which the original "leafy-gall" specimens of *S. retusus* were obtained, 50 % or more of plants of this species had been affected each year for a considerable period, but the disease had never been seen on other species grown in close proximity. After inoculation with *Bact. fascians*, 90 % of *Schizanthus retusus* plants developed large leafy galls while only 40 % of inoculated *S. grandiflora* produced small fasciated outgrowths. Similarly, both under natural conditions and by inoculation *Nicotiana glutinosa* was found to be more susceptible than *N. tabacum*.

Considerable differences in varietal susceptibility have been observed in the nurseries by chrysanthemum and carnation growers. For example, "Mason's Bronze" and "November Sun" chrysanthemums are very susceptible but "W. Turner" is resistant, while among the carnations the "Spectrum" varieties are most liable to develop the disease.

INOCULATION EXPERIMENTS

In Part I of this series (Lacey, 1936 *a*) details were given of the successful inoculation of sweet pea seedlings with strains of *Bact. fascians* isolated from growths on sweet peas, chrysanthemums, carnations, *Schizanthus* and "cauliflower" strawberry plants. Numerous further inoculations of sweet peas and of several of the other host plants have been made with these strains and, also, with more recently isolated cultures.

(1) *Sweet pea inoculations*

A routine procedure has been established for the isolation and rapid identification of *Bact. fascians* from diseased tissue.

Isolation plates of bouillon agar medium are inoculated with macerated material suspended in sterile water, colonies resembling *Bact. fascians* are subcultured on to bouillon agar and these subcultures are inoculated on to germinated sweet pea seeds in sterile bottles containing wet sand. A strongly virulent colony will produce marked fasciation in 10–14 days, but with weaker strains a month or more may elapse before infection is evident. This difference in pathogenicity was very marked in isolations made from *Nicotiana glutinosa* and *N. tabacum* leafy galls. Six colonies from each isolation were inoculated on to sweet pea seedlings; two of the *N. glutinosa* colonies induced fasciation in 14 days, four in 18 days, and all six gave strongly positive results in

3 weeks. In contrast, none of the seedlings inoculated with the *N. tabacum* colonies showed any sign of infection until the twenty-second day, when two were feebly positive; a third colony produced slight fasciation after one month and the remaining three were avirulent. *N. tabacum* is more resistant to "leafy-gall" disease than *N. glutinosa*; both under natural conditions and as the result of inoculation fewer and smaller galls are produced on the former than on the latter. These facts suggest some correlation between the weaker virulence of the *N. tabacum* strain of *Bact. fascians* and the stronger resistance of the host plant.

In the majority of isolations from the various host plants only a very few of the colonies tested failed to produce fasciation of sweet pea seedlings, but in certain cases all the colonies tested were avirulent to sweet peas. Physiological study has failed to detect any cultural differences between the virulent and avirulent strains.

Tilford (1936) obtained both "rough" and "smooth" colony types of *Bact. fascians* from his original sweet pea isolations. The writer has obtained only "smooth" colonies in original isolations but "rough" colonies developed in some cases under certain cultural conditions, e.g. in cultures subjected to drying and to the action of weak antiseptics. These "rough" colonies, with "smooth" types from the same plates, were tested on sweet pea seedlings, but no difference in virulence was detectable, both types producing equally severe fasciation.

(2) *Inoculation of chrysanthemum cuttings*

In October 1935 chrysanthemum cuttings from six varieties known to be susceptible to "leafy gall" were planted in pots, the soil of which was watered with cultures of various strains of *Bact. fascians*. Table I gives the results of these inoculations.

Table I. *Inoculation of chrysanthemum cuttings with strains of* Bact. fascians

Chrysanthemum variety	Uninoculated control		Inoculated with chrysanthemum strain 356		Inoculated with sweet pea strain 16	
	No. inoculated	No. diseased	No. inoculated	No. diseased	No. inoculated	No. diseased
Mason's Bronze	3	0	2	2	3	3
Red Admiral	3	0	2	2	3	1
Edith Cavell	3	0	2	0	2	1
Edward Page	2	0	2	1	2	2
May Wallace	2	0	2	1	.	.
Favourite	3	0	2	2	.	.
Total no.	16	0	12	8	10	7

Table I (*cont.*)

Chrysanthemum variety	Inoculated with carnation strain 353		Inoculated with *Schizanthus* strain 355		Inoculated with strawberry strain 358	
	No. inoculated	No. diseased	No. inoculated	No. diseased	No. inoculated	No. diseased
Mason's Bronze	2	2	2	2	2	2
Red Admiral	2	2	2	1	3	3
Edith Cavell	2	1	.	.	.	.
Edward Page	2	0	3	2	3	3
May Wallace	2	0	3	1	.	.
Favourite	2	2	.	.	2	0
Total no.	12	7	10	6	10	8

Ten weeks after inoculation one "Mason's Bronze" cutting inoculated with the carnation strain had developed fasciated basal shoots and, a month later, infection was evident in several of the cuttings inoculated with the strawberry, sweet pea and chrysanthemum strains. The cuttings inoculated with the *Schizanthus* strain did not produce hypertrophied shoots until 6 months after inoculation, when the controls were still free from disease. A few new infections occurred among the inoculated plants until nine months after inoculation, by which time most of the earlier produced galls had rotted. All the inoculated "Mason's Bronze" cuttings developed "leafy gall", this variety being the most susceptible of those tested.

In a second test twenty chrysanthemum cuttings (var. "Debutante") taken from healthy plants were planted in pots of sterilized soil. Five cuttings were left uninoculated, five were infected by watering the soil with a culture of the carnation strain (353) and five each with two new strains—the Shasta daisy strain 386 and the *Lilium regale* strain 388. Six weeks later several of the inoculated cuttings had developed hypertrophied basal shoots and, at the end of 2 months, three out of the five infected with the carnation strain and all those infected with the daisy and *Lilium regale* strains were diseased. Pl. XVIII, fig. 1 is a photograph of one of these cuttings taken 3 months after inoculation; all five of the controls were then still free from disease and remained so until the experiment was concluded 2 months later.

Since these preliminary tests a number of further inoculations of chrysanthemum cuttings, chiefly of varieties "Mason's Bronze" and "Debutante", have been made, either to determine the virulence of newly isolated strains of *Bact. fascians* or in connexion with experiments to control "leafy gall". In all, of 156 cuttings inoculated with strains of *Bact. fascians*, 101 developed "leafy gall". With one exception the control

cuttings in these experiments remained free from disease. In this case the cuttings were taken from plants that had had "leafy gall" but had apparently recovered, and three out of eight of the uninoculated control cuttings developed "leafy gall" from 9 to 14 weeks after planting; all the inoculated cuttings were positive 4–8 weeks after inoculation. The shortest time that elapsed between inoculation and the appearance of hypertrophied shoots at soil level was 1 month, when the cuttings were taken in the early spring (as in the usual nursery procedure). Cuttings taken in the autumn or late spring developed the disease more slowly and in fewer numbers.

In one nursery where "leafy gall" was rife the variety "W. Turner" was entirely unaffected. Nine cuttings of this variety were inoculated with *Bact. fascians* with negative results, thus confirming the field observation that "W. Turner" is markedly resistant to "leafy gall".

(3) *Inoculation of carnation cuttings*

Carnations are not so susceptible to "leafy gall" as chrysanthemums. In one nursery, where the disease has been present for 10–12 years, the grower stated that only about 5% of the carnations were seriously affected each year, the "Spectrum" variety being especially liable to attack.

In October 1935 forty carnation cuttings (variety "Salmon's Spectrum") were infected by watering the soil round the cuttings with *Bact. fascians* strains from sweet pea, carnation, chrysanthemum, *Schizanthus* and strawberry, eight cuttings being inoculated with each strain and another eight left uninoculated as controls. No definite sign of disease was apparent until May 1936 when a typical moss-like leafy gall appeared just above soil level on one cutting inoculated with the carnation strain. The gall was loosely attached to the stem by a thin stalk arising from a node 1 in. above the base of the cutting. Two of the cuttings inoculated with the *Schizanthus* strain showed similar, but smaller, growths. No further results could be obtained as, about this date, the plants were so badly damaged by cockroaches that the majority died. In a repeat experiment the following October twenty cuttings of "Salmon's Spectrum" were planted in sterilized soil. Sixteen of these were inoculated with *Bact. fascians* strains from carnation, sweet pea, *Schizanthus* or Shasta daisy and four were left uninoculated. In January three out of four of the cuttings inoculated with the sweet pea strain and one of those inoculated with the Shasta daisy strain had developed "leafy gall" and, later, one cutting inoculated with the carnation strain was

affected (Pl. XVIII, fig. 2). The controls and the cuttings inoculated with the *Schizanthus* strain remained free from disease. In a third experiment two out of four cuttings inoculated with a recently isolated chrysanthemum strain of *Bact. fascians* developed "leafy gall".

Although the number of positive infections (ten out of sixty) was low, the incidence of "leafy gall" among carnations inoculated with *Bact. fascians* was three times as great as that observed in the nursery.

(4) *Inoculation of* Schizanthus

(a) *Schizanthus retusus.* Of twenty young plants of *S. retusus* grown in sterilized soil and inoculated by pricking the stems at soil level with needles smeared with cultures of *Bact. fascians*, eighteen developed "leafy gall", infection being apparent in several of the plants 3 weeks after inoculation. In other experiments cultures were watered into the soil round young *Schizanthus retusus* plants, which were not wounded. Fourteen out of twenty-two of these plants developed "leafy gall". The incubation period was slightly longer and the percentage of positive infections somewhat lower than in plants treated by prick inoculations, but the presence of wounds is evidently not essential for the infection of *S. retusus* by *Bact. fascians*. Strains of *Bact. fascians* isolated from *Schizanthus*, sweet pea, chrysanthemum, strawberry and *Nicotiana glutinosa* were equally virulent to *Schizanthus retusus*.

(b) *Inoculation of* Schizanthus penatus *varieties.* Twenty-five young plants of *S. grandiflora* hybrids, grown in sterilized soil, were inoculated with *Schizanthus* strains of *Bact. fascians* by needle pricks at soil level. After 1 month three plants showed infection and later ten out of the twenty-five developed small leafy galls. The remaining fifteen and all the controls were negative. Two plants of *Schizanthus penatus*, grown in the nursery from which the original specimens of "leafy gall" *S. retusus* were obtained, were inoculated by needle pricks. One developed a leafy gall, the other was unaffected. Galls had never been seen on *S. penatus* plants grown in the nursery, where a large percentage of the *S. retusus* plants were affected each year, but it is evident that *S. penatus* is not entirely immune to the disease.

(5) *Inoculation of* Nicotiana *sp.*

Twenty-six young plants of *Nicotiana glutinosa* were inoculated with nine different strains of *Bact. fascians* by pricking the stems at soil level with needles smeared with culture. Twenty-five developed large leafy galls, the first signs being apparent eighteen days after inoculation.

Most of the plants were so seriously affected that they were greatly stunted, with very small, malformed leaves (Pl. XVIII, fig. 3). Six control plants developed normally. In a second experiment cultures of *Bact. fascians* were watered into the soil around eleven *Nicotiana glutinosa* plants, which were not wounded. All developed leafy galls as rapidly as those inoculated by needle pricks, thus proving that wounds are unnecessary for infection to take place.

Nicotiana tabacum. Of twenty-eight *N. tabacum* plants inoculated by needle pricks twenty-four developed leafy galls, but these remained small and did not affect the normal growth of the plant. Only one of three plants infected by watering the soil without wounding produced a very small gall. These results conform with the incidence of leafy galls under natural conditions, where, as previously noted, galls are produced much more freely and are of greater size on *N. glutinosa* than on *N. tabacum*.

(6) *Inoculation of* Heuchera sanguinea

Three young plants of *H. sanguinea* were inoculated with a *Bact fascians* strain isolated from a *Heuchera* gall. Seventeen days later one of the plants showed definite signs of disease, the new leaves being small and deformed. After 2 months the entire crown presented a "cauliflower" appearance with tiny distorted leaves arising from a mass of galled tissue (Pl. XVIII, fig. 4). The other two inoculated plants developed a "leafy-gall" type of growth at the side of the crown, which was not itself affected. A control plant developed normally.

(7) *Inoculation of petunia*

Three inoculated petunia plants all developed large leafy galls at soil level, the growth being apparent 16–23 days after inoculation.

(8) *Inoculation of* Asparagus sprengeri

During April and June twelve seedlings of *A. sprengeri* were infected by watering the soil round the base of the plants with strains of *Bact. fascians* isolated from *Asparagus sprengeri*, chrysanthemum, petunia or sweet pea. No abnormal growth developed until October, by which time three of the plants had produced typical galls at soil level and during November three more plants became diseased.

(9) *Inoculation of* Pisum sativum, Phaseolus vulgaris *and* Vicia Faba

Strains of *Bact. fascians* from sweet pea, carnation, Shasta daisy or *Lilium regale* were watered into the soil and over the apical buds of thirty-five pea seedlings. Three out of nine of the plants infected with

the carnation strain and one infected with the Shasta daisy strain developed fasciated basal shoots similar to those produced on infected sweet peas; the rest gave negative results.

Twenty French bean seedlings were inoculated with various strains of *Bact. fascians* by placing drops of water infected with the culture on the growing point between the apical leaves and also in the axils of the first pair of leaves. With two exceptions, all the plants developed galls at the apical point and in the axils of the leaves, and normal growth was completely checked. Leaves which opened after the inoculation were distorted and mottled, with raised dark green patches interposed among sunken yellowish-green areas, giving a mosaic appearance. Inoculations made by infecting the soil at the time of planting were not so successful, for out of twenty-eight plants only one was seriously affected. This developed several fasciated shoots which ceased growth when not more than 3 in. high and a definite gall was produced at soil level. Twenty of thirty-five broad bean plants, growing in soil which was infected with *Bact. fascians* at the time of planting the seeds, developed hypertrophied basal shoots of the "leafy-gall" type.

Negative inoculations. Inoculation of tomato plants by soil infection, stem pricks and by smearing the apical and axillary buds with cultures of *Bact. fascians* all gave negative results. Typical galls were produced on the stems of tomato plants by prick inoculations with *Pseudomonas tumefaciens* under the same conditions. Inoculation of potato tubers also gave negative results.

Thirty gladiolus corms were inoculated 1 month after planting by pricking the organisms into the young shoots and also by infecting the soil. No abnormal growths developed. The galls on the original gladiolus specimens had developed from the buds which normally produce the little cormels, the main corm being unaffected (Lacey, 1936 b, Pl. XXXIII, fig. 2). These cormels are formed rather late in the season and a probable explanation of the negative results is that the inoculation was made too early. The soil conditions were evidently unfavourable for the survival of *Bact. fascians*, since attempts to reisolate the pathogen from the exterior of the new corms and from the soil surrounding them, failed.

The inoculation of strawberry plants for the production of the "cauliflower" disease will be dealt with in a later paper.

A summary of the inoculation experiments is given in Table II.

Strains isolated from various host plants have been tested in these inoculation experiments to determine if there was any biological variation in pathogenicity among the strains. Four strains which were non-

Table II. *Summary of inoculation experiments with* Bact. fascians

Plant inoculated	Method of inoculation	No. inoculated	No. positive	% positive
Chrysanthemum:				
(a) Susceptible varieties	Cuttings in infected soil	156	101	65
(b) Resistant "W. Turner"	Cuttings in infected soil	9	0	0
Carnation	Cuttings in infected soil	60	10	16·6
Schizanthus:				
(a) *S. retusus*	(1) Prick inoculation	20	18	90
	(2) Infected soil	22	14	64
(b) *S. penatus*	Prick inoculation	27	11	41
Nicotiana:				
(a) *N. glutinosa*	(1) Prick inoculation	26	25	96
	(2) Infected soil	11	11	100
(b) *N. tabacum*	(1) Prick inoculation	28	24	86
	(2) Infected soil	3	1	33
Heuchera sanguinea	Prick inoculation	3	3	100
Petunia	Prick inoculation and soil infection	3	3	100
Asparagus sprengeri	Soil infection	12	6	50
Pisum sativum	Soil infection	35	4	11
Phaseolus vulgaris	(1) Inoculation of growing point	20	18	90
	(2) Soil infection	28	1	3·5
Vicia Faba	Soil infection	35	20	57
Gladiolus	Prick and soil infection	30	0	0

pathogenic to sweet peas were also avirulent to *Schizanthus retusus*, but eight strains virulent to sweet peas all produced leafy galls on *S. retusus*. Twelve strains pathogenic to sweet peas were also pathogenic to chrysanthemum cuttings, though there was some variation in the degree of infection, the length of the incubation period and the number of cuttings affected. Nine strains were tested on *Nicotiana glutinosa* and *N. tabacum*. All nine were pathogenic for both species and in every case the disease was more serious on *N. glutinosa* than on *N. tabacum*. Negative results were, however, obtained by the inoculation of *Pisum sativum* with the sweet pea and *Lilium regale* strains, and of *Vicia Faba* with the *Lilium regale* and Shasta daisy strains though these were virulent to sweet peas and chrysanthemums. The sweet pea, carnation, tobacco and chrysanthemum strains were all pathogenic to *Vicia Faba* and *Phaseolus vulgaris*.

These results show that the various strains of *Bact. fascians* have a wide host range. With the exception of the sweet pea and *Schizanthus* inoculations with avirulent strains, the number of plants inoculated in experiments in which a particular strain has failed to produce galls on any host has been too small to constitute a proof of complete avirulence to that host; further inoculations are necessary.

The examination of plants either naturally or artificially infected with *Bact. fascians* shows that the galls are invariably produced by the stimulation and abnormal proliferation of bud tissue. The plumules of sweet pea seedlings inoculated immediately after germination may become hypertrophied, but the greater part of the galled tissue develops from the lateral buds which in normal seedlings remain latent unless the plumule is injured. In plants such as *Nicotiana, Schizanthus*, etc., the leafy galls have their origin in the nodal buds at the base of the stem, which in normal plants do not develop. Chrysanthemums normally produce basal shoots from these buds and infection by *Bact. fascians* causes "leafy-gall" tissue to be formed in their stead. Buds in the axils of leaves of aerial stems may give rise to galls (*Phaseolus* sp., *Forsythia*) or the entire growing point may be involved, giving rise to the growths known as "cauliflower" (*Heuchera*, strawberry). Finally, as in gladiolus and *Lilium regale*, buds which normally develop into storage organs may be affected.

Attempts to produce abnormal proliferation of any tissues other than buds have failed. Prick inoculation of *Bact. fascians* into the internodes of young sweet pea seedlings have invariably been negative and no excess callus growth resulted from the inoculation of carrot slices kept in moist Petri dishes (a successful method of testing the virulence of *Pseudomonas tumefaciens*). Wounds are not necessary for gall formation by *Bact. fascians* but *Pseudomonas tumefaciens* has no action on an uninjured surface. Chrysanthemum cuttings may be attacked by both *Bact. fascians* and *Pseudomonas tumefaciens*, but whereas the former will produce a "leafy-gall" type of growth from the underground buds and have no effect on the cut end, the latter will cause the formation of a compact gall of undifferentiated tissue at the base of the cutting. A prick with a needle smeared with a culture of *Ps. tumefaciens* passing through the cambium in any part of an actively growing stem (e.g. tomato) will cause the formation of a wart-like gall; galls of similar appearance may be produced by infection with *Bact. fascians* (e.g. *Asparagus sprengeri*), but only at the nodes. *Bact. fascians* has no direct action on root tissue, and the roots of an infected plant are not affected unless the plant is greatly weakened by the disease.

Microscopical examinations of sections of young galls of *Schizanthus*, *Nicotiana* and chrysanthemum showed that, as in the sweet pea, the bacteria are mainly confined to the exterior of the growths. In some places a thick bacterial zoogloea was pressed against the outer walls of the epidermis, with occasional penetration into the intercellular spaces

and the formation of pockets of necrosed tissue due to the destruction of the epidermal and subepidermal cells. In the vicinity of the bacterial zoogloea active cellular division of the surrounding tissue caused the production of irregular protuberances in precisely the same manner as that seen to occur in inoculated sweet pea seedlings.

EVIDENCE THAT *BACT. FASCIANS* MAY BE SEED-BORNE

Tilford (1936) states that *Bact. fascians* may be carried on the exterior of the seed coat of sweet peas. The history of "leafy gall" on *Schizanthus retusus* suggested that, in this plant also, the disease might be seed-borne. In the nursery from which the original specimens were obtained the disease had appeared every spring for a number of years on *S. retusus* plants, of which more than one-half were invariably infected. The disease usually developed rapidly after the plants had been potted when about 6 in. high. Soil sterilization had failed to check the disease and, as the potting soil was imported fresh each year from various parts and no variation in the amount of disease was noticeable from year to year, the soil would seem to be eliminated as a source of infection. The seed, however, was collected indiscriminately from both healthy plants and those not too seriously affected by the disease to produce normal flowers.

In the spring of 1936 seeds of the 1935 crop of *S. retusus* from the infected nursery were planted in pots of sterilized soil. Fifty-four seeds germinated and 2 months after planting one of these showed definite signs of disease, the hypocotyl being greatly swollen and the first leaves small and yellow. Numerous colonies of *Bact. fascians* were obtained on isolation plates made from the hypocotyl tissue. Later, another seedling developed a leafy gall from which *Bact. fascians* was isolated.

In a second experiment a number of seeds of the 1936 crop of *Schizanthus retusus* from the infected nursery were embedded in bouillon agar. The seeds became covered with a mixed bacterial growth, amongst which, in several cases, were organisms resembling *Bact. fascians*. The mixed growth from twelve of the seeds was inoculated on to sweet pea seedlings, two of which developed fasciations from which *Bact. fascians* was isolated. The percentage of infected seeds in these samples was evidently small, but with even one diseased seedling in a seed box the infection might become widespread through watering or by the contamination of the hands or tools during transplanting.

PHYSIOLOGICAL TESTS

A study of the physiological reactions of forty strains of *Bact. fascians* has failed to disclose any means by which avirulent and virulent strains can be distinguished in culture. The work has, however, yielded some interesting results. It was stated in Part I of this series (Lacey, 1936 *a*) that while the production of acid from sugars in synthetic media was very slow and feeble (being determined by the change in colour from blue to yellow of brom-thymol-blue indicator in the medium), yet this afforded some means of differentiation between the strains, in that the carnation, chrysanthemum and strawberry strains produced acid from lactose and the *Schizanthus* and sweet pea strains did not. By a continuation of these tests the various strains of *Bact. fascians* were divided into two main groups—those that produced acid from lactose and non-lactose fermenters. These were each separated into two sub-groups (*a*) producing slight acid from mannitol, (*b*) causing no increase in the hydrogen-ion concentration of mannitol media. All the strains produced some acid from glucose and saccharose in varying degrees. Virulent and avirulent strains occurred in each group but in most cases the strains isolated from a particular host plant were identical in cultural reactions. For example, the *Asparagus sprengeri* strain from Germany and the two English strains were in close agreement not only in failing to produce acid from lactose but also in the amount of the increase of the hydrogen-ion concentration from day to day of the glucose, saccharose and mannitol cultures. Similarly, a strain isolated from a pelargonium gall from Germany was identical with the English strains from the same host. The strawberry and *Heuchera* strains, all of which were isolated from " cauliflower " growths, were in group II *b* (acid from lactose, but not from mannitol) and a sweet pea strain, isolated three years after the original isolations were made, was identical with the latter. An exception was found in the chrysanthemum strains, for of the six cultures tested, three produced acid from lactose and three did not; all six were virulent on sweet pea.

Eight strains (sweet pea, asparagus, *Schizanthus*, pelargonium, carnation, chrysanthemum, dahlia and strawberry) were tested in a number of other carbohydrate synthetic media. All eight produced acid from levulose, galactose and mannose, and slightly increased the hydrogen-ion concentration in the xylose and maltose tubes, but had no action on dulcite, dextrin, sorbitol and inulin. The first six strains had a feeble action on arabinose, while the dahlia and strawberry strains were negative, and the pelargonium strain alone produced acid from rhamnose.

Liquefaction of gelatine

In the first paper of this series (Lacey, 1936 *a*) and according to Tilford (1936), *Bact. fascians* was found to be a non-liquefier of gelatine at 20° C. Further tests have shown that, when the cultures were incubated at 24° C., liquefaction began at the surface of a stab culture in 2–4 weeks and the medium gradually became of a thick treacly consistency. This change did not occur in gelatine stabs at 24° C. inoculated with, e.g., *Pseudomonas fluorescens non-liquefaciens.* (A similar type of liquefaction was observed by Burkholder (1930) to occur in gelatine cultures of *Ps. medicaginis* var. *phaseolicola.*) The organism cannot be said to be a true gelatine-liquefier, but the reaction is characteristic of this species.

Litmus milk. All the strains of *Bact. fascians* produced a deep blue coloration in litmus milk. In the majority of cultures a soft curd was slowly formed and still more slowly digested, but these reactions varied with the different batches of milk used and there was no uniformity among strains isolated from any one host plant.

Nitrate reduction. In the original description, this organism was stated to be a non-nitrate reducer, the tests having been made on nitrate broth and on liquid synthetic nitrate media. Tilford (1936), however, obtained feeble nitrate reduction on synthetic nitrate agar, but not on peptone beef-extract agar. Further tests of the strains of *Bact. fascians* isolated by the writer have confirmed Tilford's results, in that most of the strains will feebly reduce nitrate when grown on synthetic nitrate agar, but not on fluid synthetic nitrate or peptone media.

SUMMARY

1. *Bact. fascians* (Tilford), the causal agent of fasciation of sweet peas, leafy galls, etc., has been isolated from abnormal growths on plants of twenty-five different genera belonging to sixteen families, including both Monocotyledons and Dicotyledons.

2. All these strains of *Bact. fascians* were inoculated on to sweet pea seedlings which, in the majority of cases, became severely fasciated.

3. Characteristic galls have been produced on chrysanthemums, carnations, *Schizanthus* sp., *Nicotiana* sp., *Heuchera sanguinea*, petunia, *Asparagus sprengeri*, *Pisum sativum*, *Phaseolus vulgaris* and *Vicia Faba* by inoculation with various strains of *Bact. fascians*. No distinct biological variations among the strains could be detected.

4. Inoculation of gladiolus, tomato and potato gave negative results:

5. The galls are invariably produced in the region of bud tissue. In young growths the bacteria are mainly confined to the exterior of the galls.

6. The organism may be carried on the seeds of *Schizanthus*.

7. A study of the physiological reactions of forty strains of *Bact. fascians* has failed to disclose any means by which virulent and avirulent strains can be distinguished in culture.

REFERENCES

BROWN, N. A. & WEISS, F. (1937). Crown gall of the fasciated type on *Asparagus sprengeri*. *Rep. U.S. Dep. Agric. Plant Dis.* **21**, 31.

BURKHOLDER, W. H. (1930). The bacterial diseases of the bean. *Bull. Cornell agric. Exp. Sta.* no. 127, p. 48.

LACEY, M. S. (1936 *a*). The isolation of a bacterium associated with fasciation of sweet peas, "cauliflower" strawberry plants and "leafy gall" of various plants. *Ann. appl. Biol.* **23**, 302.

—— (1936 *b*). Further studies on a bacterium causing fasciation of sweet peas. *Ann. appl. Biol.* **23**, 743.

TILFORD, P. E. (1936). Fasciation of sweet peas caused by *Phytomonas fascians* n.sp. *J. agric. Res.* **53**, 383.

EXPLANATION OF PLATES XVII AND XVIII

PLATE XVII

Fig. 1. Galls on *Asparagus sprengeri* fronds. Nat. infection.

Fig. 2. "Fasciation" of nasturtium. Nat. infection.

Fig. 3. Galls on *Forsythia suspensa*. Nat. infection.

Fig. 4. Fasciated shoot of *Chrysanthemum indicum*. Nat. infection.

PLATE XVIII

Fig. 1. Chrysanthemum cutting 3 months after infection with *Bact. fascians* (*Lilium regale* strain).

Fig. 2. Carnation cutting 6 months after infection with *Bact. fascians* (carnation strain).

Fig. 3. *Nicotiana glutinosa* seedling 6 weeks after infection with *Bact. fascians* (sweet pea strain).

Fig. 4. *Heuchera sanguinea* infected with *Bact. fascians* (*Heuchera* strain). Photographed 4 months after inoculation.

Fig. 1.

Fig. 2.

Fig. 1.

Fig. 2.

Fig.'3.

Fig. 4.

LETTUCE MOSAIC

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(With Plates XIX and XX)

CONTENTS

INTRODUCTION

Mosaic disease of lettuce was first described in Florida, U.S.A., by Jagger (1921) and later by Brandenburg (1928) in Germany and by Ogilvie (1928) in Bermuda. Ogilvie & Mulligan (1931) were the first definitely to record the disease in England, where mosaic has since been noted by Pethybridge *et al.* (1934), and, on lettuce under glass, by Ainsworth (1937). It is probable that all these investigators studied the same disease but the causal virus has never been described, and the purpose of this paper is to place on record a description of the virus and a more detailed account of the disease than has hitherto been published.

METHODS

Most of the field observations were made in the west of England, particularly in the Evesham market garden area and on experimental plantings at Long Ashton, while the work involving experimental

inoculations was carried out under controlled conditions under glass at Cheshunt.

Lettuce plants for inoculation experiments were raised from virus-free seed in an insect-free glasshouse, and for making routine tests seedlings growing in 3 in. pots were used. The variety Cheshunt Early Giant, a cabbage lettuce of the Gotte group, was employed almost exclusively as the test plant.

The method of inoculation finally developed was to grind infected leaves with a little 0·5 % sodium sulphite solution and, after the addition of a small quantity of powdered carborundum, inoculate leaves by gentle rubbing with a pad of butter muslin moistened with inoculum, when 80–100 % infection of lettuce resulted.

For the experiments on insect transmission suitable insect-proof cages were employed.

SYMPTOMS

The symptoms on mature cabbage lettuce (*Lactuca sativa* L. var. *capitata* Hort.) in the field, at the time when normal plants are ready for cutting, comprise dwarfing, defective hearting, and mottling or yellowing, necrosis or scorching, and distortion of the leaves. The mottling and yellowing symptoms are most noticeable during the spring and necrosis and scorching during the summer. Clearing of the veins is a common symptom in both young and old plants, and in addition there may be an irregular pale blotching, or the whole leaf may simply be rather yellow and hard looking, as if injured by wind or frost. The leaves may also be more blistered or ballooned than usual and have their shape further altered by emphasis of the serrations so that the characteristic features of a variety may become disguised. Necrosis takes the form of numerous minute necrotic brown spots between the veins or a more definite vein necrosis, and severe scorching of the leaf edges may occur. The effect of mosaic is particularly noticeable when the plants "bolt". A diseased plant is stunted, the leaves on stem and inflorescences show a pronounced mottle and sometimes necrosis, while in certain varieties necrotic lesions develop on the stem, the bracts, and the flower buds (Pl. XX, fig. 2 and cf. Brandenburg (1928), p. 45, Abb. 11), when flowers may fail to open. The amount of seed is reduced. In general, diseased flowering plants may be distinguished by their yellow colour, scorched appearance and shortness.

Under experimental conditions in the glasshouse the first symptom, which appears 7–14 (usually about 10) days after inoculation by either

mechanical means or insects, is a pronounced clearing of the veins of the younger leaves (Pl. XIX, fig. 2). Later, the vein-clearing becomes either less pronounced, when a more general mottling of the leaves is seen, or more pronounced so that a definite necrosis of the veins occurs (Pl. XIX, fig. 1). Affected plants are usually paler in colour than corresponding healthy plants, satisfactory hearting may be interfered with, and there is a greater or less degree of stunting (Pl. XX, fig. 1). Most of the symptoms observed in the field have been reproduced experimentally, but an important factor for symptom expression in cabbage lettuces is the variety infected. This is dealt with in the succeeding section.

Inoculations made under glass at different times of the year have indicated little seasonal effect on either the incubation period or the general symptoms, but, in the case of Cheshunt Early Giant, the variety used most extensively, vein necrosis tended to be replaced in mid-winter by severe yellowing of the veins. The severity of the symptoms shown by an individual plant is to some extent correlated with its size when inoculated; a seedling plant being more badly stunted, etc., than a plant inoculated at a later stage of development, and this effect is also evident in the field.

In cos lettuce (*L. sativa* L. var. *romana* Hort.) the first symptom is a clearing of the veins, and subsequently a more or less pronounced mottle develops (Pl. XX, fig. 4). Vein necrosis is rare, but scorching of the leaf edges tends to occur and the plants are stunted (see Ogilvie *et al.* 1935, p. 183, plate 2, fig. 2). Infected plants fail to make a compact heart (Pl. XX, fig. 3), and this symptom is useful in distinguishing diseased and healthy plants when the mottling is rather indefinite. Several varieties of cos lettuce have been inoculated in the glasshouse but there has been little variation in the symptoms and a similar uniformity has been observed in the field.

Although mosaic diseased plants can usually be recognized without difficulty in the field, the differences in varietal reaction combined with varying environmental conditions and the effects of aphis infestation, unfavourable soil conditions and root rots may, especially on outdoor winter lettuce, make the certain diagnosis of mosaic impossible without recourse to inoculation tests.

VARIETAL REACTIONS OF CABBAGE LETTUCES TO MOSAIC

It was evident from the observations of Ogilvie & Brian (1936) that there were considerable differences between the reactions of different

varieties of cabbage lettuce to mosaic. In order to examine this point twenty-two different varieties of cabbage lettuce were raised from seed under the same conditions in a heated glasshouse, and about five plants of each variety were inoculated at the five-leaf stage and, subsequently, five more plants of each variety at the eight- to nine-leaf stage. The plants were growing in 3 in. pots when inoculated but, later, infected and healthy plants of both series were transferred to 6 in. pots and kept under observation for approximately 2 months (March–May) after inoculation. These tests are summarized in Table I. When the lettuce varieties employed were grouped according to Brian's (1936) classification it was found possible to make certain generalizations concerning the symptoms exhibited by the members of the different groups. With a few exceptions groups 2, 3, 4, 6 and 8 were characterized by mottle symptoms while in groups 7, 5 and 1 the necrotic type of symptom was severe. (In the notes which accompany Table I the reactions of lettuces of the different groups when inoculated under experimental conditions are set out parallel with the observations made on the behaviour of the same types in the field.)

Little evidence was found to indicate any differences in susceptibility between any of the varieties tested, but the symptoms ranged from the severely necrotic type exhibited by Whitsuntide (Pl. XIX, fig. 1) to the mild mottling exhibited by members of the Trocadero and Crisp (Pl. XIX, fig. 3) groups.

It will be seen that while the inoculation tests have shown large differences in the varietal reaction of cabbage lettuces to mosaic, various discrepancies exist between the estimate of the general severity of the disease under experimental conditions and the estimate for field conditions.

It must be remembered that most of the lettuces inoculated experimentally were types not intended to be forced under glass (see the last column of Table I) and that the estimate of disease intensity in the field has a commercial bias. For example, a variety mildly attacked as judged by the general symptoms would be classed as severely affected in the field if unsatisfactory hearting resulted when the lettuce was grown at its appropriate season.

(1) *Gotte*

Experimental. Moderately severe mottle, moderate to severe vein necrosis, and rather severe stunting were typical of this group (Pl. XX, fig. 1). Resistant Early French Frame was less badly affected than the other two varieties of the Gotte group tested, the mottle was slight and there was very little necrosis, and this variety and

Table I. *Reaction of cabbage lettuces to mosaic*

Group (Brian's classification)	Variety	% infection*	Symptoms	General reaction Experimental inoculation	General reaction Field estimate	Type of lettuce
(1) Gotte	Resistant Early French Frame	20	m, n (trace), ms, S	Severe	—	F
	Tennis Ball B.S.	40	M, n, S	Very severe	Severe	F
	Cheshunt Early Giant†	80	M, n–N, ms, S	Severe	—	F
(2) All the Year Round	All the Year Round	90	m, n (trace), s	Severe	Severe	Sp, S (W)
	Feltham King	80	M, n (trace), s	Severe	Severe	Sp, S (W) (F)
	Spring Beauty	100	M, s	Severe	Severe	Sp, S, W
(3) May Queen	May King	100	M, s	Severe	Severe	F, Sp
(4) Trocadero	Trocadero	90	m, s (slight)	Mild	Mild	Sp, W
	Improved Trocadero	78	M, n (trace), s	Severe	Severe	S, W
	Premium	90	m–M, n (trace), s	Mild	Mild	S, W
	Unrivalled	89	M, n (trace), s	Mild	Severe	Sp, S, W
	White Boston	78	m, n (trace), s	Mild	—	Sp, S, W
(5) Lee's Immense	Lee's Immense	100	M, n–N, ms, S	Very severe	Mild	W
	Harrison's Green Winter	80	m, n, MS, s	Severe	Mild	W
	Yates's Winter White	60	M, n (trace), s	Severe	Severe	W
(6) Passion	Arctic (Sutton)	90	M, ms, s	Severe	Severe	W
	Arctic (Hurst)	90	M, s	Severe	Severe	W
	Arctic King	100	M, s	Severe	Severe	W
	Imperial	100	M, n–N, ms, S	Very severe	Severe	W
	Stanstead Park	100	m–M, s	Mild	Mild	W
(7) MacHattie's Giant	Whitsuntide	90	M, N, S	Very severe	Severe	W
(8) Crisp	Iceberg	100	m–M, s (slight)	Mild	Severe	S
	Webb's Wonderful	77	m–M, s (slight)	Mild	Severe	S

Lettuce type: Sp, Spring
S, Summer
W, Winter
F, Forcing

Symptoms: M, Vein-clearing, mottle
N, Vein necrosis
MS, Marginal scorch
S, Stunting

(Capitals, symptoms intense; small letters, symptoms less well developed.)

* Average $\frac{188}{226} = 83.5\%$.

† Cheshunt Early Ball reacts in a similar manner.

Tennis Ball B.S. showed the two lowest percentage infections of the whole series. The low percentage infections may perhaps be accounted for by the rather leathery leaf texture shown by these varieties, which makes artificial inoculation more difficult. Once infected, however, plants of these two varieties showed little tolerance to the virus.

Field observations. In the Gotte class symptoms are severe. The plants are greatly dwarfed and mottled or yellow. Necrosis is not very marked. The leaves of the flower stalks are greatly reduced in size.

(2) *All the Year Round*

Experimental. A mild to moderately severe mottle was a predominating symptom of this group.

Field observations. In the pale varieties of the All the Year Round group the symptoms are severe. If infected at an early stage the size of the plant is considerably reduced. The leaves are yellowed or mottled with dark areas near the veins and the rest of the leaf pale, more blistered than usual and with necrotic spots between the veins. There may be marked scorching of the leaf edges in the summer. The leaf serration may be greatly emphasized.

In the dark green All the Year Round varieties the symptoms are slight, only slight hardening of the outer surfaces of the leaves and slight scorching and slight failure to heart.

In the tinted All the Year Round varieties the leaves assume a brownish colour.

In Spring Beauty and Feltham King the plants are so slightly affected as usually to be saleable.

(3) *May Queen*

Experimental. May King, which was the only representative of the group tested, showed a very pronounced yellowing of the veins as the early symptoms (Pl. XIX, fig. 2) and, later, a general mottle and stunting, but no necrosis.

Field observations. In the May Queen group the leaves are conspicuously mottled or yellow in colour, the plants are stunted and hearting is poor.

(4) *Trocadero*

Experimental. Lettuces of this group proved rather tolerant to the virus. Trocadero showed a mild mottle without necrosis and slight stunting while Improved Trocadero was rather more severely attacked. The remaining varieties were intermediate between these two types.

Field observations. In the Trocadero group there is marked blistering of the leaf. An accentuation of the serration of the leaf edges alters the appearance of the lettuce considerably. The affected plants are paler than normal, but hearting is usually little affected.

(5) *Lee's Immense*

Experimental. Mottle, necrosis and stunting were all pronounced. Necrosis was most severe in Lee's Immense and least severe in Yates's Winter White. Harrison's Green Winter showed particularly severe marginal scorch.

Field observations. In the Lee's Immense group the plants are usually small, pale and slightly mottled, while hearting may be only slightly or considerably reduced.

(6) *Passion*

Experimental. As a group these varieties were characterized by a well-developed mottle, or very slight necrosis, and slight stunting. Imperial was an exception and showed rather severe necrosis and more pronounced stunting.

Field observations. In the Arctic types the plants are greatly dwarfed. The leaves are pale or mottled and much crinkled. There is little or no hearting.

On the flowering stem of Passion varieties grown in summer numerous elongated brown necrotic spots may occur. These coalesce and lead to a general browning or to elongated lesions which eventually may split with exudation of latex. There is also a necrosis of the small leaves of the flowering stem.

In the Stanstead Park types there is only a slight paling of the leaves, with little reduction in hearting. Normally the leaves are smooth, but in mosaic-infected plants they are often markedly corrugated. The leaflets on the flower stems show clearing of the veins and are irregularly blotched, the pale areas being mostly concentrated towards the centres of the leaflets. Small necrotic spots may be found in the pale areas. When at the edge of the leaflet these may coalesce into a marginal scorching.

(7) *MacHattie's Giant*

Experimental. Whitsuntide, the only variety of the group tested, showed the most severe necrotic symptoms of the whole series (Pl. XIX, fig. 1).

Field observations. In the MacHattie's Giant group the plants are greatly dwarfed and the leaves very crinkled and mottled.

(8) *Crisp*

Experimental. Iceberg and Webb's Wonderful showed a mild to moderately severe mottle with slight stunting and no necrosis, and were the most tolerant varieties tested (Pl. XIX, fig. 3).

Field observations. In the Crisp varieties clearing of the veins is very evident. The outer surfaces of the leaves assume a hard appearance. Hearting is poor and there is a tendency to shoot prematurely.

HOST RANGE

Experimental inoculations, supplemented by field collections, have extended the host range from lettuce to other members of the Compositae and also to certain leguminous plants.

Attention was paid to certain composite weeds commonly found in lettuce plantings. *Senecio vulgaris* L., groundsel, was found to be susceptible and subsequently diseased specimens were collected from several localities and the presence of the virus proved by inoculation tests. The symptoms shown by groundsel are very mild. About 10 days after inoculation there is a definite, but transitory, clearing of the veins and, later, by careful comparison with healthy plants, a faint mottle can at times be observed. Infected plants are not noticeably stunted, they flower profusely and there is some evidence of seed transmission

(see below, p. 288). *Sonchus asper* Hoffm., prickly sow-thistle, has also been infected and shows a definite leaf mottle but *S. oleraceus* L. could not be infected and no diseased specimens were found in collections of this species and of *S. arvensis* L. *Taraxacum officinale* Web. and *Carduus arvensis* Curt. could not be infected.

It was found that both *Lathyrus odoratus* L., sweet pea, and *Pisum sativum* L., garden pea, were susceptible to lettuce mosaic virus. In sweet pea the virus causes a streak disease while in garden pea a mild mosaic mottling results. In sweet pea there is no apparent reaction for 3–4 weeks, or longer, when severe streak symptoms appear in the stems, usually towards the base, but the initial streaks may be 6 in. or more above soil level, and some shoots may wither and die. If the shoot is not killed the apical leaves remain healthy in appearance or possibly at times show a very mild mottle, but contain a high concentration of the virus. The first symptom in garden pea (var. Lincoln) is a mild vein-clearing about 2 weeks after inoculation and, subsequently, a very mild rather blotchy mottle develops on some leaves of certain plants, but the general effect on this variety of pea has been slight. After experiments had proved that these plants are susceptible naturally infected plants of both sweet pea and garden pea, the former showing streak symptoms, were found in the field. The question of the relation of lettuce mosaic virus to the streak disease of sweet peas in this country is at present under investigation.

Attempts to infect *Lycopersicum esculentum* Mill. (tomato), *Nicotiana tabacum* L. var. White Burley (tobacco), *N. glutinosa* L., *Datura Stramonium* L., *Cucumis sativus* L. (cucumber) and *Brassica oleracea* L. var. *Botrytis cymosa* (cauliflower) failed.

The virulence of the virus to lettuce is in no way reduced by passage through groundsel or garden pea, in which the symptoms are mild.

TRANSMISSION

(a) *By seed*

Newhall (1923), in Western New York, was the first to demonstrate that lettuce mosaic was seed transmitted. In two trials, he obtained 4·8 and 3·0 % average transmission by sowing seed saved from individual mosaic diseased plants of the variety Big Boston and he found 1–3 % of mosaic diseased seedlings in field plantings examined at the two- to three-leaf stage. Brandenburg (1928) claimed 8–10 % transmission for Punktmosaik while Ogilvie & Mulligan (1934) and Ogilvie *et al.* (1935) recorded 22–37 % infected plants in sowings of seed from mosaic-diseased

cos lettuce (as compared with 11% in sowings of commercial seed). Ogilvie's estimates were made on unprotected field plants and therefore included both seed transmission and secondary infection caused by insects.

In order to examine the question of seed transmission, seed, saved from individual mosaic-diseased plants, was sown in seed boxes containing sterilized soil at the rate of fifty seeds per box. Particular care was taken to insure freedom from insects and the seedlings were examined at frequent intervals and any diseased plants at once carefully removed. The sowings were made at intervals during 1937 (see Ainsworth, 1938) and 1938 and the results, including the numbers of control seedlings raised, are summarized in Table II, from which it can be seen that seed transmission occurred in each of the three varieties employed.

Table II. *Seed transmission of lettuce mosaic*

Variety	No. seedlings raised	No. infected seedlings	% infection
Trocadero	952	56	5·6 (a)) *
	667	55	8·3 (b))
	275	10	3·64
Total	1894	121	6·40
Feltham King	667	41	6·15 (a)) *
	550	40	7·3 (b))
	64	1	1·57
Total	1281	82	6·4
Lobjoit's Dark Green Cos	276	6	2·17
Grand total	3451	209	6·06
Controls†	862	0	0

(a) 1-year-old seed. (b) 2-year-old seed.

* Difference between these numbers not significant.
† In addition, no case of mosaic occurred in the hundreds of seedlings raised for experiment from virus-free seed in the same glasshouse.

No symptoms of disease appeared on the cotyledons but diseased seedlings were slightly stunted and could be recognized by a distinct mottling of the second, or third, and subsequent foliage leaves. To prove that the seedlings were attacked by mosaic, healthy lettuce plants were inoculated from diseased seedlings, when they developed typical mosaic symptoms, and tests showed the virus obtained from the seedlings to be similar to that with which the seed plants were infected. It was usual to grow the plants on in the boxes to the seven- to nine-leaf stage, but sometimes representative samples were transferred to pots and grown to a larger size. No plant which appeared healthy at the four- to five-leaf stage was observed to develop mosaic.

Portions of certain samples of the seed saved from infected plants in 1936 were sown in the spring and summer of 1937 as 1-year-old seed, and further portions during 1938 as 2-year-old seed. There was no diminution in the amount of transmission through the 2-year-old seed (see Table II) and mosaic seedlings have been found in sowings of commercial seed known to be at least 3 years old.

An attempt was made to ascertain whether the virus was also transmitted through groundsel (*Senecio vulgaris*) seed. A total of 846 plants was raised from seed saved from experimentally infected groundsel plants, and, because of the difficulty in recognizing mosaic disease in groundsel, 530 of these plants (mostly taken at random) were tested for the presence of virus by the inoculation of juice of individual plants into one to three lettuce seedlings or by inoculating a group of lettuce plants with inoculum prepared by grinding together a number of whole groundsel seedlings or separate leaves from larger plants, with or without sodium sulphite solution. The inoculation tests revealed the presence of three infected groundsel plants and, although so few infected plants were detected, the result is thought to be significant.

Several hundred seedlings were raised from seed taken from infected *Sonchus asper* plants but all appeared healthy.

(b) *By insects*

General field observations indicate that spread of lettuce mosaic is associated with infestation by aphides. Jagger (1921) was able to transmit the virus by means of *Myzus persicae* Sulz. and Brandenburg (1928) transmitted Punktmosaik by *Macrosiphum hieracii* v.d.G. At Long Ashton, in 1934, two lots of fourteen lettuce plants were placed under cages in the open and, to each plant of one lot, two *Macrosiphum sonchi* L. were transferred from a mosaic diseased plant on 22 June. On 19 July three of the fourteen plants showed symptoms of mosaic, whilst the fourteen control plants under the other cage were all healthy. Dr K. M. Smith has informed the writers (*in litt.*, July 1938) that he succeeded in transmitting the virus by *Myzus lactucae*, but that this insect did not appear to be a very efficient vector as only about a quarter of the experimental plants developed the disease.

To supplement these observations experiments were performed to test the ability of several different aphides to transmit the virus under controlled conditions. The aphides, kindly identified by Mr F. Laing, Mr E. R. Speyer and Dr C. L. Walton, were either collected from

naturally infested plants, e.g. *Myzus persicae* from carnations, or stocks were raised from a few selected individuals. In most experiments the aphides were starved for an hour or two before feeding on a diseased plant (as advised by Watson, 1936) and the feeding time was relatively short, the insects being caged 15–25 min. on diseased plants, although in some experiments the time on infected plants was extended to 72 hr. The insects were next caged for 24–30 hr. on healthy plants which were then fumigated, set aside, and kept under observation. Five to ten aphides were placed on each test plant. Several independent tests were made with most aphides and the results are summarized in Table III.

Table III. *Transmission of lettuce mosaic by aphides under controlled conditions*

	No. plants inoculated	No. plants infected
Myzus persicae Sulz.	33	15
M. pseudosolani Theob.	35	0
M. hieracii Kalt.	18	0
Macrosiphum gei Koch.	23	1
M. sonchi Linn.	46	0
Rhopalosiphum rufomaculatum Wilson	9	0

Myzus persicae transmitted the virus in each of four independent tests (20–90 % transmission), but, of the other aphides used, only *Macrosiphum gei* gave any positive results. The negative results are not sufficiently extensive to disprove the ability of these aphides to act as vectors of lettuce mosaic virus in the field. They only indicate that the aphides in question do not transmit the virus efficiently under conditions favourable for its transmission by *Myzus persicae*. An indication of the efficiency of aphides as vectors in the field is shown by the following experiment. On 30 June ten seedling lettuce plants growing in pots were caged in the field with several mosaic diseased cos lettuces which were infested with aphides. These were mostly *M. hieracii*, but a few individuals of other species may have been present. Ten days later there were very few aphides on the caged plants owing to the activities of parasites, but during the period 18–25 July seven of the ten seedlings developed symptoms of mosaic. The remaining three plants, on which no aphides were found, were transferred to the glasshouse where they remained healthy.

Aphides occurring on lettuces. In the case of the following records of aphides occurring on lettuces the insects were identified by Dr C. L. Walton.

Macrosiphum gei Koch. is by far the commonest aphis on lettuces in

the Bristol Advisory Province. It was found, for example, on lettuces on the following occasions:

Late summer, 1930.	At Long Ashton.
29 March 1932.	At Long Ashton.
15 Oct. 1934.	Fairly numerous throughout the Province.
May 1935.	Very abundant at Charlton, near Evesham, and associated with severe mosaic.
9 Oct. 1935.	Sparsely on seed beds at Evesham. Distinctly mottled plants at this date.
15 Oct. 1935.	On seed beds at Fishponds, Bristol.
19 Nov. 1935.	Very prevalent at Perdiswell, Worcs.
22 May 1936.	Very common at Fladbury, near Evesham.

Macrosiphum sonchi Linn. was found on lettuces as follows:

22 June 1934.	At Long Ashton.
18 May 1938.	Very common on young summer lettuces at Cheltenham.

Myzus lactucae Schr. was found on lettuces as follows:

May 1933.	At Charlton, Worcs.
29 Oct. 1935.	Considerable infestation on old plants of summer lettuces at Cheltenham.
Nov. 1935.	At Perdiswell, Worcs.
4 Mar. 1936.	In glasshouses, Long Ashton.

Myzus persicae, the most efficient transmitter of the disease under controlled conditions, has not been found on lettuces in the Bristol Advisory Province, but was recorded on *Sonchus oleraceus* at Long Ashton on 9 December 1933. Mr C. T. Gimingham, of the Ministry of Agriculture's Plant Pathological Laboratory, informs the writers that *Myzus persicae* is rarely recorded on lettuce in other parts of the country.

Myzus pseudosolani Theob. has been found infesting lettuce under glass in Hertfordshire. The saliva of *M. pseudosolani* is toxic to lettuce seedlings and leaves on which this aphis has fed become very malformed.

Other aphides are occasionally recorded on lettuces.

It must be concluded from the above records that *M. persicae* is not the principal vector of lettuce mosaic in Great Britain and, although the fact that aphides spread the disease in the field is established, the elucidation of the conditions under which transmission occurs requires further investigation.

FIELD OBSERVATIONS

In the course of the Vegetable Disease Survey carried out by Long Ashton Research Station during recent years it has been found that mosaic disease commonly causes considerable loss in lettuce plantings. In Worcestershire the name "rust" has long been applied by growers to a complex of diseases which includes mosaic disease, ring spot (caused by

Marssonina Panattoniana) and *Botrytis* disease. Of these three diseases mosaic is by far the most important.

Presumed causes. The disease has been attributed by growers in the past to faulty manuring, to deficiencies of essential chemicals in the soil, to mixed seed, giving rise to "rogues", and to the direct effects of aphis infestation.

Economic importance. During years when the disease is prevalent it may cause great loss in winter lettuces in the south-west of England. Entire fields may become worthless and have to be ploughed under, and this may be reflected in scarcity of lettuces on the market.

In the spring of 1934 many plantings of winter lettuces failed entirely in the Bristol, Cheltenham and Evesham districts. Counts were made of diseased plants in twenty-four commercial plantings with the following results:

> Bristol: av. 34 % infection
> Cheltenham: av. 46 %   ,,
> Evesham: av. 39 %   ,,

In the spring of 1935, when mosaic was again prevalent, similar counts gave the following results:

> Bristol: av. 39 % infection
> Cheltenham: av. 57 %   ,,
> Evesham: av. 38 %   ,,

Seasonal occurrence. Mosaic disease does not usually occur to any marked extent on greenhouse lettuces. Here and there occasional affected plants may be found, but unless aphides are present, which is usually not the case in well-managed greenhouses, there is little spread.

In the west and south-west of England winter lettuces are sown out of doors in August and September to stand the winter. They make little growth during the winter months but grow rapidly during the early spring and are ready for cutting during May.

A few diseased plants may usually be found among the seedlings in the early autumn. These arise, presumably, from infected seeds. It is probable that much spread takes place at this time if the weather is dry and aphides are prevalent. This is indicated by the fact, well known to growers, that much less disease becomes apparent if the seeds are sown later in the open, or in frames in January or February before planting out. Thus in one case at Cheltenham an early autumn planting gave at maturity 66 % infection, a late autumn planting 35 % infection and a February planting (from frames) 20 % infection.

In the west of England the disease is not usually common on the Trocadero and other varieties grown throughout the summer. This is, apparently, because the numbers of aphides soon become reduced by parasites and predators. In seasons when aphides are very prevalent during the summer, however, mosaic disease may be prevalent on summer lettuces also. For instance, in August 1938, 75 % mosaic was recorded on one variety of summer lettuces at Long Ashton.

Relation to weather conditions. Prevalence of mosaic disease in spring on winter hardy varieties of lettuces is usually correlated with dry weather, favourable to the multiplication of aphides, during the previous October and November when the plants were in the seedling stage. This is seen from the data assembled in Table IV, and it is interesting to note that these observations are in line with those of Davies (1935) on the effect of humidity on the activities of aphides on potatoes.

Table IV. *Rainfall at Long Ashton and incidence of mosaic on winter lettuces in the Bristol Advisory Province*

	Rainfall in in. (Oct. and Nov.)	Incidence of mosaic in the following spring
1932	10·50	Slight
1933	3·41	Very prevalent (for % see above, p. 291)
1934	3·53	Very prevalent (for % see above, p. 291)
1935	12·53	Slight (about 5%)
1936	6·71	Slight (about 5%)
1937	5·37	Slight

The average total rainfall for October and November during the last 25 years was 7·43 in.

PROPERTIES OF THE VIRUS

The properties of the virus were, with the exceptions noted, determined with preparations of crude lettuce sap prepared by grinding infected lettuce plants in a mortar and then expressing the sap through muslin. Powdered carborundum was added to the preparations before inoculating the test plants.

Resistance to heat. Samples of infected juice were enclosed in thin-walled test-tubes and subjected to various temperatures for 10 min. periods. The results are summarized in Table V, from which it can be seen that the virus is inactivated at a temperature between 55 and 60° C.

Table V. *Resistance of lettuce-mosaic virus to heat*

	Unheated	50° C.	55° C.	60° C.	70° C.
No. of plants infected	27*/39	10/56	1/37	0/47	0/10
% infection	69·3	17·9	2·7	0·0	0·0

* Denominator, number of plants inoculated; numerator, number infected.

Resistance to ageing. The results of the tests on the longevity of the virus are summarized in Table VI.

Table VI. *Resistance of lettuce-mosaic virus to ageing* in vitro

Time in hr.	0	6–7	12	24	48	72
No. of plants infected	36*/69	5/48	2/20	7/63	1/48	0/35
% infection	52·2	10·4	10·0	11·1	2·1	0·0

* Denominator, number of plants inoculated; numerator, number infected.

In crude juice the virus survives 48 hr. or less when stored *in vitro* at laboratory temperature. It was detected after 24 hr. in three of seven tests and after 48 hr. in one of five. The rate of inactivation of the virus in expressed sap is rapid. Sap from a mosaic-diseased lettuce was used to inoculate ten lettuce seedlings as soon as it was expressed and then further sets of ten plants were inoculated at intervals of 1, 2, 3 and 6 hr. All the plants of the first set developed mosaic, while in the later series the numbers of infected plants were four, one, two and two respectively. It was thought that, perhaps, the inactivation of the virus might be delayed in the presence of a reducing agent, as is the virus of tomato spotted wilt (Bald & Samuel, 1934; Ainsworth, 1936). It was found that if juice was prepared by grinding the infected material with a little 0·5 % sodium sulphite solution the virus could regularly be detected after 24 hr. (up to 80 % infection) and more frequently after 48 hr. It was not detected in preparations 3 days old or older. It is, however, probable that the virus could be preserved *in vitro* for longer periods under appropriate conditions.

Filtration. The virus could not be filtered through a thin layer of Kieselguhr or Celite or through an L 1 Pasteur-Chamberland filter, even when filtration was carried out quickly and in the presence of sodium sulphite.

Dilution. The amount of dilution the virus will stand is low. It has been detected at 1/50 with juice diluted with water and at 1/100 if diluted with sodium sulphite solution, while higher dilutions gave negative results. A rough estimate of the virus concentration in plants infected for different periods of time was made by inoculating on one occasion series of ten lettuces with preparations of equal concentration on a fresh weight basis, of plants 14–90 days after inoculation, but no difference in virus concentration was detected.

DISCUSSION

The virus here described has been detected in mosaic diseased lettuces obtained from different localities in this country and has, so far, been the only virus found attacking lettuce in England. The disease is almost

certainly the same as that described by Jagger (1921), and an indication
that the American lettuce mosaic is identical with the one found in this
country is afforded by the fact that the same virus was found infecting
one mosaic diseased seedling which appeared in a sowing of a commercial
sample of Big Boston lettuce seed obtained direct from an American
seed company. Brandenburg (1928) described two types of mosaic,
Blattnervenmosaik and Punktmosaik. The former was seed transmitted
to the extent of approximately 20% and could not be transmitted by
either mechanical means or aphides, while the latter was transmissible
by seed (8–10%) and insects, but no mention is made of its transmission
by mechanical methods. It is probable that lettuce mosaic should be
identified with Punktmosaik, with which it agrees in general symptoms
and behaviour, but the dot mosaic symptom has been less evident than
under Brandenburg's conditions. No disease that can be equated with
Blattnervenmosaik has been observed but, in certain varieties, lettuce
mosaic appears as a vein-banding very similar to that of Blattnerven-
mosaik (cf. Pl. XIX, fig. 2 with Brandenburg (1928), p. 40, Abb. 2
and 3).

The status of the virus has not yet been fully elucidated, and the
question of its relation to the already described leguminous viruses is
under investigation. It is probable that the host range could be extended
but the hosts recorded in this paper, together with the properties of the
virus described, should make future recognition of the virus possible.
The virus is quite distinct from that of tomato spotted wilt, which also
attacks lettuce (Tompkins & Gardner, 1934) and causes a streak disease
in sweet pea (Snyder & Thomas, 1936). Spotted wilt has not yet been
recorded on lettuce in Great Britain but experimental inoculations have
shown the symptoms to be necrotic local lesions (Pl. XX, fig. 5 A) which,
if not the only reaction, are followed by a systemic necrosis of the central
leaves (Pl. XX, fig. 5 B), often one sided as described by Tompkins &
Gardner (1934), without mosaic mottling. Systemic infection in seedling
lettuce has resulted in the death of the plants.

CONTROL MEASURES

Lettuce mosaic can be controlled on the lines applicable to most virus
diseases. As healthy as possible a stock of seedlings must be raised and
steps taken to ensure that the virus is not spread within the crop or
introduced from without. When devising methods for the control of
lettuce mosaic it must be remembered that the virus is seed transmitted,
aphides are vectors, certain common weeds are susceptible to the disease,

and different varieties of cabbage lettuce vary greatly in their reaction to the virus.

Provided virus-free seed is used, or the seedlings are carefully examined before being transplanted, there appears to be no reason why mosaic should prove a serious disease, difficult to control, in glasshouses and frames where aphides can be eradicated relatively easily. It is in the field, where aphis attack is more difficult to combat, and in situations where weeds are allowed unchecked that mosaic becomes a serious disease. Under such conditions the chief points to which attention must be paid are as follows:

(1) *Clean seed.* Virus-free seed should be sown. No doubt in time seedsmen and growers of lettuces for seed will recognize the importance o roguing out diseased plants and reaping seed from healthy plants only The feasibility of doing this has been shown in this paper.

(2) *Choice of seed bed and planting ground.* It has been found that less aphis infestation and less mosaic disease is apt to result in hilly and open situations. In low-lying situations surrounded by trees and buildings, infection is likely to be severe, as can be seen from the percentage infection at Cheltenham in comparison with other localities given above (p. 291).

(3) *Time of sowing.* In the case of winter lettuces sown outside to stand the winter, fairly late sowings and sowings in frames tend to reduce aphis infestation and, hence, to give a cleaner crop. Planting out in spring also appears to give less mosaic infection than otherwise.

(4) *Use of insecticides.* The use of nicotine sprays or dusts would no doubt reduce aphis infestation, but the value of these in controlling spread of mosaic infection out of doors is problematical. Nicotine fumigation in glasshouses is effective in reducing aphis infestation.

(5) *Weeds.* Weeds should be eradicated. Special attention must be paid to groundsel for, although not a perennial plant, overlapping generations of this weed occur all the year round, especially in gardens.

(6) *Choice of varieties.* It is recommended that in localities where the disease is troublesome, varieties which show only mild symptoms might be substituted for those which become markedly affected. Where, however, the market value of the lettuce is important, care should be taken to select a variety suitable in colour, size and time of maturing, and one which will grow satisfactorily under the conditions in question.

In Table VII the characters and uses of the different groups of cabbage lettuces are briefly summarized and the reactions of a number of varieties indicated.

Table VII. *Response of lettuce groups to mosaic disease*

Group	Characters	Use	Varieties which show severe symptoms	Varieties which show slight symptoms
(1) Gotte	Leaves and heart rounded	Forcing	Loos Tennisball	—
(2) All the Year Round	Round, leaves rather smooth, green	Open (spring and summer)	All the Year Round, Feltham King	Exceller, Spring Beauty
(3) May Queen	Leaves blistered, hearts loose, lower leaves flat	Forcing, Frames, Open (spring)	May Queen, May King, Winter Victor	—
(4) Trocadero	Hearts good, flat on top. Lower leaves flat. Edges of mature leaves folded	Open (summer)	Unrivalled, Improved Trocadero	Trocadero, Premium, Improved Unrivalled, All Seasons Perfection
(5) Lee's Immense	Leaves dull, smooth edges, yellowish	Open (winter)	Yates's Winter White, Schofield's Winter White, Hardy Winter Green	Hardy Hammersmith, Webb's Immense Hardy Green, Clarke's Imperial, Green Winter, Lee's Immense
(6a) Passion	Compact, hardy	Open (winter)	Arctic, Arctic King, Imperial, Majestic, Excelsior	—
(6b) Stanstead Park	Large, spreading. Brown spots on leaves	Open (winter)	—	Stanstead Park, Early Spring, Winter Tremont
(7) MacHattie's Giant	Large, spreading, blistered leaves	Open (winter)	MacHattie's Giant, Whitsuntide, Goliath, Best of All	—
(8) Crisp	Leaves curled and blistered	Open (summer)	Webb's Wonderful, Summer Standwell	—

SUMMARY

Previous records of lettuce mosaic are cited. The symptoms of the disease on lettuce are described, and the variation in the reaction of different varieties of cabbage lettuce indicated. The host range is extended to certain composite weeds (including groundsel, *Senecio vulgaris*) and to members of the family Leguminosae (*Pisum sativum*, *Lathyrus odoratus*). Experiments in which the virus was seed transmitted through lettuce to the extent of about 6% are described, and the results of experiments with aphis vectors reported. An account of field observations on the influence of the weather on the disease is given. Certain properties of the virus are described and the status of the virus is discussed. Control measures are suggested.

REFERENCES

AINSWORTH, G. C. (1936). Detection of spotted wilt in chrysanthemums. *Nature, Lond.*, **137**, 868.

—— (1937). *Rep. exp. Res. Sta. Cheshunt*, 1936, p. 60.

—— (1938). Lettuce mosaic. *Rep. exp. Res. Sta. Cheshunt*, 1937, p. 54.

Fig. 1.

Fig. 2.

Fig. 3.

AINSWORTH AND OGILVIE.—LETTUCE MOSAIC (pp. 279–297)

BALD, J. G. & SAMUEL, G. (1934). Some factors affecting the inactivation rate of the virus of tomato spotted wilt. *Ann. appl. Biol.* **21**, 179–90.

BRANDENBURG, E. (1928). Über Mosaikkrankheiten an Compositen. *Forsch. PflKr., Berl.*, **5**, 39–72. (See RATHSCHLAG, *Obst- u. Gemüseb.* **75**, 114–15, 1929, 4 figs., for a summary of Brandenburg's results.)

BRIAN, P. W. (1936). Varieties of cabbage lettuce and their classification. *J. Pomol.* **14**, 26–38.

DAVIES, W. MALDWYN (1935). Studies on aphides infesting the potato crop. III. Effect of variation in relative humidity on the flight of *Myzus persicae* Sulz. *Ann. appl. Biol.* **22**, 106–15.

JAGGER, I. C. (1921). A transmissible mosaic disease of lettuce. *J. agric. Res.* **20**, 737–9.

NEWHALL, A. G. (1923). Seed transmission of lettuce mosaic. *Phytopathology*, **13**, 104–6.

OGILVIE, L. (1928). *Rep. Dep. Agric. Bermuda*, 1927, p. 31.

OGILVIE, L. & BRIAN, P. W. (1936). *Rep. agric. hort. Res. Sta. Bristol*, 1935, p. 110.

OGILVIE, L. & MULLIGAN, B. O. (1931). *Rep. agric. hort. Res. Sta. Bristol*, 1930, p. 134.

———— (1934). *Rep. agric. hort. Res. Sta. Bristol*, 1933, p. 112.

OGILVIE, L., MULLIGAN, B. O. & BRIAN, P. W. (1935). *Rep. agric. hort. Res. Sta. Bristol*, 1934, p. 183.

[PETHYBRIDGE, G. H., MOORE, W. C. & SMITH, A.] (1934). Fungus and other diseases of crops, 1928–32. *Bull. Minist. Agric.* no. 79, p. 58.

SNYDER, W. C. & THOMAS, H. R. (1936). Spotted wilt of the sweet pea. *Hilgardia*, **10**, 257–62.

TOMPKINS, C. M. & GARDNER, M. W. (1934). Spotted wilt of head lettuce. Abst. *Phytopathology*, **24**, 1135–6.

WATSON, M. A. (HAMILTON) (1936). Factors affecting the amount of infection obtained by aphis transmission of the virus Hy. III. *Philos. Trans.* B, **226**, 457–89.

EXPLANATION OF PLATES XIX AND XX

PLATE XIX

Fig. 1. Cabbage lettuce, var. Whitsuntide, showing symptoms of mosaic 17 days after inoculation. (Healthy control plant on right.)

Fig. 2. Cabbage lettuce, var. May King, showing symptoms of mosaic 16 days after inoculation. (Healthy control plant on right.)

Fig. 3. Cabbage lettuce, var. Iceberg, showing symptoms of mosaic 36 days after inoculation. (Healthy control plant on right.)

(The photograph for Fig. 1 was taken with a green filter to emphasize the necrosis and that for Fig. 2 with a red filter to emphasize the yellow vein-clearing, hence the apparent large variations in leaf colour.)

PLATE XX

Fig. 1. Cabbage lettuce, var. Cheshunt Early Giant, showing symptoms of mosaic 36 days after inoculation. (Healthy control plant on left.)

Fig. 2. Necrotic lesions on stem and bracts of a "bolted" Cheshunt Early Giant plant.

Fig. 3. Cos lettuce, var. Lobjoit's Dark Green Cos, showing symptoms of mosaic. (Healthy control plant on left.)

Fig. 4. Cos lettuce, var. Demi Plate Maraîchère, showing mosaic symptoms.

Fig. 5. Spotted wilt on lettuce, var. Cheshunt Early Giant: A, local lesions 10 days after inoculation; B, systemic necrosis after 18 days.

EXPERIMENTS AND OBSERVATIONS ON A VIRUS DISEASE OF WINTER SPINACH (*SPINACIA OLERACEA*)

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(With Plate XXI)

CONTENTS

INTRODUCTION

DURING the winter of 1936 the writer's attention was drawn to a disease of winter spinach occurring at Slough. Reports from growers indicated that the disease was prevalent in the neighbourhood, and that the intensity varied from season to season.

Winter spinach is sown from the third week in August till about the first week in October, and is usually left until the following spring, when picking commences and continues till the middle of May. The earliest sowings may be given one or more pickings before the winter sets in and then left until the spring.

Plots were laid down in the autumn of 1936 to ascertain if the condition was due to a mineral deficiency. Two plots were treated with sulphate of potash at the rate of approximately 4 cwt./acre, two with borax at the rate of 20 lb./acre, and two were left untreated as controls. The amount of disease on the various plots bore no relation to the treatment, and on investigating the problem along different lines it was found

that a virus disease was concerned. It is not to be assumed that the diseases of spinach noticed from time to time by growers are all of this nature: waterlogging of the soil and mineral deficiencies may play a part.

HISTORICAL

The *List of Common Names of British Plant Diseases* (1929) contains no reference to a virus disease of spinach. Dillon Weston (1934) records the occurrence of spinach mosaic in Bedfordshire. The virus nature of the disease was confirmed by Dr K. Smith (quoted by Dillon Weston, 1935), but no indication is given as to the relationship of the virus principle concerned.

A virus disease of spinach, called "spinach blight", was first recorded from America by McClintock & Smith (1918). It is characterized by a mottling and malformation of the leaves, by a decided stunting of growth and by premature death of the plant. The disease is transmissible by needle inoculations and by aphides. Boning (1927) has recorded the presence, in the neighbourhood of Bonn, of a disease very similar to the above and shown that the virus is transmissible to mangolds by insects but not by mechanical inoculation. Volk (1929) describes the occurrence of an epidemic of spinach mosaic in the foothills between Bonn and Cologne, losses in many places amounting to 90–100 %. The general impression amongst German workers is that the disease recorded from Germany is identical with the American disease, but the description given by Boning is slightly different from that recorded by McClintock & Smith. No attempt was made to identify the virus responsible for the disease in Germany.

Hoggan (1929) showed that the virus causing cucumber mosaic is transmissible to spinach and that the symptoms produced are very similar to those described for spinach blight. Later (1933) she demonstrated that spinach is susceptible to at least three different viruses, namely those of cucumber and sugar-beet mosaic, and the tobacco ringspot virus. The first two are frequently responsible for the death of the plant. The symptoms produced by sugar-beet mosaic on spinach differ from those produced by cucumber mosaic in that there is no fine discrete spotting of the leaves as a primary symptom, and no malformation of the leaves occurs later. As a result of her investigations Hoggan considers that the American spinach blight is due to cucumber mosaic virus. This result is also supported by the isolation of the virus from naturally diseased material, whilst she tentatively suggests on the basis of the published descriptions that the German disease is due to the virus of

sugar-beet mosaic. Wilhelm (1935), however, showed that the virus responsible for the mosaic of spinach in the neighbourhood of Bonn is transmissible to cucumber and vice versa. Furthermore there is no record of sugar-beet mosaic infecting cucumbers.

Smith (1937) records infection of spinach by sugar-beet mosaic, sugar-beet curly-top virus and *Callistephus* virus. Tobacco-mosaic virus has been described by Jones (1934) as infecting spinach and killing the plant.

<div style="text-align:center">DESCRIPTION OF THE DISEASE</div>

During the winter of 1936-7 observations on the disease were made at Slough, on the plots mentioned. Individual plants showing early symptoms were selected and observations made on the course of the disease until the death of the plant. At any time throughout the winter, plants showing all stages of the disease could be found and Pl. XXI, figs. 1 and 2 show typical examples of diseased and healthy plants obtained from the field and potted.

The first visible symptom of the disease in the field is a yellowing of the younger leaves, which becomes more pronounced, and spreads to the outer leaves as the plants become older. At this stage the plant becomes stunted and the young leaves are distorted, their edges being cut and feather-like. The outer leaves become limp, and the tips lie on the ground. Eventually, the whole leaf lies on the ground, by which time the outer leaves have lost most of their green colour. The last part of the leaf to remain green is usually that bordering the veins (Pl. XXI, fig. 2 B).

Disintegration of the older tissues begins, starting at the tips of the leaves, and working its way inward until only the petioles remain (Pl. XXI, fig. 1 D, E). The plant is reduced to a small clump of much distorted leaves 1-2 in. in diameter, and later the rotting spreads to the inner leaves and the plant dies. During the winter of 1936-7 the rotting of the tissues was much more rapid than in 1937-8, when owing to an exceptionally dry spell the progress of disintegration was delayed and the leaves became brown and shrivelled. The root of the plant remains in healthy condition for a considerable time, and begins to disintegrate only during the final stages of the disease.

The time taken for these changes to occur varied from about 50-64 days after the appearance of the first symptoms. In experimentally inoculated plants the time from inoculation to death varied from 80 to 96 days, the first symptoms appearing about 20-30 days after inoculation.

An attempt was made to obtain seed from diseased plants, but the majority of them had died before the time of flowering. Some plants which became infected later in the season sent up short distorted shoots, and in one or two plants seed was set. However in most cases the embryo was small and shrivelled.

Inoculation studies

The cause of the disease and the relationship of the active principle were determined as follows:

Spinach. (a) Twelve spinach plants, chosen at random in a field crop which had been sown in the previous August, were inoculated in February by rubbing juice from diseased plants on to the leaves with the pestle which had been used for grinding up the tissues. Twenty-four plants, one on either side of those inoculated in the row, were similarly rubbed with water to serve as controls. Eleven of the former developed typical symptoms of the disease, whereas all the controls appeared healthy after three months.

(b) Twelve plants which had been grown in the greenhouse were inoculated as in the previous experiment, twelve others serving as controls. Apart from a tendency to develop the flower axis, due to the forcing conditions in the greenhouse, the inoculated plants all developed the typical symptoms. Control plants remained healthy.

(c) Seedling spinach plants in the greenhouse were inoculated at the time when the first two rough leaves had developed. Of thirty-two such plants, fifteen developed the disease. The infected plants showed yellowing and malformation of the younger leaves and finally died. Pl. XXI, fig. 3 illustrates a typical example of a diseased and a control plant 8 weeks after inoculation.

Cucumber. Inoculation of young cucumber plants by rubbing their surfaces with sap from diseased spinach gave positive results with about one-half of the plants tested. Infected plants became stunted and the young leaves developed a yellow mottle. This mottling is characterized by the formation of dark green raised areas and the whole leaf, therefore, gives the impression of abnormal greenness. There is some distortion of the leaves and the internodes of the stem are reduced. Fruit is set less readily than in the control plants and diseased fruits show a yellow mottle.

Tobacco. The symptoms were very mild and showed only when the plants were rapidly growing. The first symptom was a slight clearing of the veins, followed by a mild mottle.

Nicotiana glutinosa. The symptoms were more definite than those produced on tobacco. The leaves became mottled and somewhat distorted. In one plant necrotic lesions were produced at the tip of some of the younger leaves. The plants became stunted.

Tomato. No mottling or distortion of the leaves could be detected, and the only symptom was an abnormal tendency for the lateral buds to develop.

No successful inoculations were obtained with expressed sap which had been stored in the laboratory for 2 days.

Of the six viruses which are known to produce symptoms in spinach four may be eliminated in the case of the disease under consideration.

Sugar-beet curly-top virus is not easily sap-inoculable, and *Callistephus* virus I can be transmitted by insect vectors only. The symptoms produced by the spinach virus on tobacco and *Nicotiana glutinosa* differ from those described by Smith (1937) as typical of tobacco mosaic and tobacco ring-spot virus. The former produces local lesions on *N. glutinosa* whilst the latter gives ring-like patterns on tobacco. The causal agent must, therefore, be the virus of sugar-beet mosaic or of cucumber mosaic or an unrecorded virus. Table I gives a comparison between sugar-beet mosaic, cucumber mosaic I, and the spinach virus.

Table I. *Comparison of sugar-beet mosaic, cucumber mosaic I and spinach virus*

	Sugar-beet mosaic	Cucumber mosaic I	Spinach virus
Longevity *in vitro*	24–48 hr.	72–96 hr., 48–72 hr. (Ainsworth, 1935)	24–48 hr.
Transmission	Transmitted by hard rubbing	Easily transmitted by sap	Easily transmitted by sap
Spinach	Yellow flecks on young leaves, no marked malformation; necrosis of outer leaves followed by death of plant	Uniform chlorosis and malformation of young leaves; necrosis of older leaves followed by death of plant	Uniform chlorosis and malformation of young leaves; necrosis of outer leaves followed by death of plant
Tobacco (mechanical inoculation)	Not infected	Slight clearing of veins followed by mild mottle	Slight clearing of veins followed by mild mottle
Nicotiana glutinosa	Not infected	Mild mottle and distortion of leaves; stunting of plant	Mild mottle and distortion of leaves; stunting of plant
Cucumber	Not infected	Yellow mottle and distortion of leaves; stunting of plant; fruit mottled	Yellow mottle and distortion of leaves; stunting of plant; fruit mottled
Tomato	Not infected	Mild mottle without distortion and slight narrowing of the leaves; "Fern leaf" produced under certain conditions; Mogendorff (1930) records production of lateral leaflets and shoots	No leaf distortion or mottle; tendency for lateral shoots to develop

There is thus a great similarity between the virus of cucumber mosaic and that of the spinach disease. The only differences noted were that the spinach virus had a shorter period of longevity, and that rather different symptoms were produced on tomato. However, the period of longevity is of the same order, and it was shown by Ainsworth (1935) that

the longevity of cucumber mosaic was rather variable. In only two out of eight tests recorded by Ainsworth was the virus active on the third day.

The symptoms produced on tomato by cucumber mosaic have also been shown to vary considerably. "Fern-leaf" symptoms, according to Mogendorff (1930), are produced only occasionally when the virus is inoculated artificially into tomato plants. The prolific growth of the axillary buds and the branching of the main stem are described by him as being caused by the virus, but neither Ainsworth (1935) nor Smith (1937) records these symptoms.

Inoculations were carried out with the virus obtained from spinach and with authentic cucumber mosaic I obtained from Dr Ainsworth. The viruses were inoculated on to spinach, tobacco, *Nicotiana glutinosa* and cucumber. Identical symptoms were produced on the last three hosts, but spinach tended to flower so rapidly, with consequent withering of the leaves, that no definite symptoms developed.

The results of the above inoculation studies indicate that the disease is caused by cucumber mosaic I, and that it is probably identical with the disease recorded by McClintock & Smith (1918) from America.

FIELD STUDIES

Observations were made during the season of 1937–8 on the occurrence of the disease in the field. Various growers in the Thames Valley were visited and the disease was found to be fairly severe at three centres. Estimates of the intensity of the disease were made by taking sample counts, and the loss due to the disease was shown to be 19, 13 and 40–50 % of the total stands at the three farms. The following factors appear to be important in the incidence of the disease.

(a) *Time of sowing*

In the autumn of 1937 an experiment was carried out at Slough to ascertain if the date of sowing affected the severity of the disease. Sowings were made on 4 August, 18 August and 1 September, four plots (10 × 10 ft.) being sown on each occasion. The plots were situated in one field, and the replicates were randomized as far as the position of the plots would allow. Table II gives the number of diseased plants present on the plots, at intervals of two months, throughout the season. Germination was good in all the sowings, but the final stand on some of the earliest sown plots was poor owing to the date of sowing being too early, so that many plants flowered before the winter set in. Frost also killed some of the earliest sown plants.

Table II. *Effect of date of sowing on the occurrence of the disease*
at Slough, 1937–8

		No. of diseased plants on			
Plot no.	Date of sowing	6 Oct.	13 Dec.	10 Feb.	10 April
1	4 Aug.	1	7	12	19
2	,,	5	8	20	7
3	,,	2	12	15	27
4	,,	37	54	*	*
5	18 Aug.	1	0	2	0
6	,,	0	0	2	5
7	,,	0	2	8	12
8	,,	0	0	2	8
9	1 Sept.	0	0	0	0
10	,,	0	0	0	0
11	,,	0	0	0	0
12	,,	0	0	8	2

* Plants affected by frost.

Table II shows the progress of the disease as the winter advanced. No account was taken of plants which had died and disappeared, and this explains the low numbers of diseased plants present in some plots on the last date of recording. Table II also shows that the earlier the sowing the greater the incidence of disease.

(b) *Aphis infestation*

Samples of seventy plants taken from each plot were examined for the presence of aphides. The results are given in Table III, which shows the numbers of plants infested with winged and wingless forms.

Table III. *Aphis infestation on* 15 *September* 1937

		No. of plants infested with aphides		
Plot no.	Date of sowing	Winged	Wingless	% infestation
1	4 Aug.	2	8	14
2	,,	8	6	20
3	,,	1	9	14
4	,,	4	35	54
5	18 Aug.	1	0	1·5
6	,,	1	6	10
7	,,	1	3	6
8	,,	1	5	8·5
9, 10, 11, 12	1 Sept.	0	0	0

Table III indicates a higher degree of aphis infestation in the earlier sowings, and a comparison with Table II shows that aphis infestation is correlated with the amount of disease present on the plots. Plot 4, which had the highest percentage infestation, developed

most disease, whilst the last sowings showed no aphis infestation and negligible disease. The aphis population declined rapidly after the middle of September, and very few could be found on the spinach at Slough from November onwards, and into the late spring. This would account for the relatively small amount of spread which was shown in the Slough plots during the winter. On the other hand, at three of the farms visited, the disease continued to spread throughout the winter, chiefly along the rows where the plants were in contact. At two centres there was also a considerable amount of lateral spread, so that diseased patches up to 3–4 ft. in diameter were seen. These showed at the edges plants in various stages of the disease, whereas in the centre all traces of the plants had gone. At these farms there was in the spring a considerable infestation of the crop with aphides, and it may be presumed that a certain population of aphides had overwintered on the crop.

(c) Sources of infection

In order that a crop may become infected by means of aphides, there must be a source of virus inoculum in its vicinity. This may consist of seedlings derived from virus-infected seed or of one or other of the alternative host plants of the virus.

Seed-borne transmission. Seed was collected from diseased and healthy plants during the summer of 1937. Great difficulty was met with in obtaining seed from infected plants, and it was only from such as had become infected in the spring that any seed was obtained. Table IV indicates the weight and percentage germination of seed from diseased and healthy plants.

Table IV. *Comparison of seed from diseased and healthy plants*

Source of seed	Weight of 100 seeds (g.)	% germination
Artificially inoculated	0·21	10
Control	0·42	85
Naturally diseased	0·26	15
Control	0·57	90

The low percentage germination of seed obtained from diseased plants materially reduces the risk of seed-borne transmission. The few plants which were raised in the greenhouse from this source showed no trace of disease after 5 weeks' growth, so that the evidence for the moment is that seed transmission does not occur. However, this possibility should not be ruled out, as the experiment was not on a sufficiently extensive scale, and it may be that diseased seed is produced only when the parent plants become infected at a definite stage in their development.

(d) *Other host plants*

Cucumber mosaic virus is known to be able to infect a large number of plants. Smith (1937) gives a list of 100 such species extending over thirty-two families. Attempts were made during the summer of 1937 to obtain naturally diseased material.

Vegetable marrows were observed' on a farm showing typical symptoms of a virus disease. Sap from these plants was inoculated into cucumber plants and typical symptoms of cucumber mosaic developed. Summer spinach was also found to be infected. These alternate hosts are probably the chief sources from which infection spreads to spinach in the autumn.

Cucumbers have been recorded from Germany as probably being the chief alternate host of the virus, and at two centres in the Thames Valley, where the disease was worst, a crop of vegetable marrows had been grown within a quarter of a mile of the winter spinach. Both crops were present in the field at the same time, and thus it would be possible for the disease to pass directly from marrows to spinach in the autumn, provided suitable insect vectors were present.

Weed hosts were also examined for the presence of virus, especially *Bryonia dioica*, but naturally infected material of the latter could not be found.

CONTROL MEASURES

In view of the wide host range of cucumber mosaic I, control of the spinach disease will depend partly on the removal of plants carrying the virus in the vicinity of this crop. Vegetable marrows have been shown to be capable of being infected, and spinach should not be planted in their neighbourhood. However, in order to establish definitely that the disease is contracted by spinach from marrow further investigations are necessary on aphis migration from marrow to spinach.

Experiments and observations show that the disease is worst in early sown crops, and an obvious method of control is to postpone the date of sowing till the latter half of September. Since the disease is readily transmissible by mechanical means, there is a serious risk of its being carried along the rows on the hands of workers. This would apply especially when—as is sometimes practised—an early sown crop is picked in the autumn and then allowed to stand over the winter for spring pickings. To compensate for the loss of the autumn picking a crop could be sown in the late summer and, after picking in the autumn, be ploughed in.

SUMMARY

A disease of winter spinach common in the Thames Valley has been shown to be of a virus nature. It is characterized by a mottling and malformation of the leaves, stunting of growth and death of the plants. The causal agent has been shown to be identical with cucumber mosaic I of Ainsworth.

The disease is most prevalent in the earlier sown crops, and this appears to be correlated with their liability to be infested with aphides. Field observations on a number of farms indicated a correlation between intensity of aphis infestation and intensity of disease.

Seed-borne infection, though possible, has not been proved.

Under field conditions in the Thames Valley it is suggested that a main source of contamination of winter spinach is the outdoor vegetable marrow crop.

Postponement of date of sowing until the latter half of September is suggested as a method of reducing the amount of disease.

REFERENCES

AINSWORTH, G. C. (1935). Mosaic diseases of the cucumber. *Ann. appl. Biol.* **22,** 55.

BONING, K. (1927). Über die wechselseitige Übertragbarkeit der Mosaikkrankheiten von Rube und Spinat. *Zbl. Bakt.* Abt. 2, **71,** 490.

BRITISH MYCOLOGICAL SOCIETY (1929). List of common names of British plant diseases. *Trans. Brit. mycol. Soc.* **14,** 39.

HOGGAN, ISMÉ A. (1930). Transmission of cucumber mosaic to spinach. *Phytopathology,* **20,** 103.

—— (1933). Some viruses affecting spinach and certain aspects of insect transmission. *Phytopathology,* **23,** 446.

JONES, L. K. (1934). Tobacco mosaic on spinach. *Phytopathology,* **24,** 1142.

McCLINTOCK, J. A. & SMITH, L. B. (1918). True nature of spinach blight and relation of insects to its transmission. *J. agric. Res.* **14,** 1.

MOGENDORFF, N. (1930). "Fern leaf" of tomato. *Phytopathology,* **20,** 25.

SMITH, K. (1937). *A Text-Book of Plant Virus Diseases.* London.

VOLK, A. (1929). Das diesjährige starke Auftreten der Mosaikkrankheit (Gelbfleckigkeit) des Spinates. *Mitt. Inst. Pflkrankh. Bonn-Poppelsdorf.* (Abs. in *Rev. appl. Mycol.* **9,** 428.)

WESTON, W. A. R. DILLON (1934). *Minist. Agric. Monthly Summary of Plant Pests and Diseases in England and Wales.* November, p. 11.

—— (1935). *Minist. Agric. Monthly Summary of Plant Pests and Diseases in England and Wales.* May, p. 12.

WILHELM, A. J. (1935). Die Gelbfleckigkeit des Spinates (Spinatkrankheit). *Obst u. Gemüseb.* **81,** 56.

EXPLANATION OF PLATE XXI

Fig. 1. Spinach, from field: A, healthy; B–E, progressive stages of disease.

Fig. 2. Spinach: A, healthy; B, naturally infected.

Fig. 3. A, spinach artificially infected in the greenhouse by rubbing leaves with sap from diseased plant; B, control.

Fig. 4. Cucumber leaves: A, control; B, inoculated with virus from spinach.

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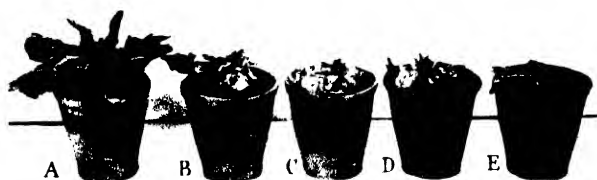
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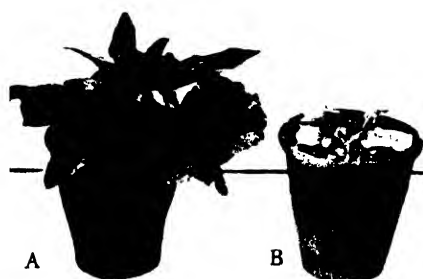
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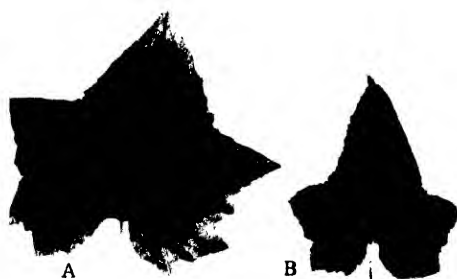
Fig. 4.

Fig. 3.

STOREY.—Experiments and Observations on a Virus Disease of Winter Spinach
(*Spinacia oleracea*) (pp. 298–308)

A GAMASID MITE (*TYPHLODROMUS THRIPSI* N.SP.), A PREDATOR OF *THRIPS TABACI* LIND.

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(With 15 Text-figures)

CONTENTS

INTRODUCTION

FOR about 10 years *Thrips tabaci* has been bred on cotton plants grown in a glasshouse at the Manchester University Experimental Grounds so that observations on the biology of this insect could be carried out. It was noticed that, under glasshouse conditions, the natural enemies of the thrips were very few. No parasites were observed and the only important predator appeared to be a gamasid mite. The first appearance of thrips on the plants occurred very regularly about the first week in May each year, and the first mites were generally observed by the end of June. After this time the mites could be obtained in fairly large numbers until the end of November, by which time the thrips had almost disappeared for the season.

The gamasid, which has been identified for me by Dr H. Graf Vitzthum as a new species of *Typhlodromus*, bred very readily under laboratory conditions. Owing to the small size of the mites, especially in the early stages, they were kept in small glass tubes, $1\cdot5 \times 0\cdot25$ in. It was most important that the corks by which the tubes were closed had no holes or cracks as the mites were able to squeeze into very small cracks and were thus liable to be transferred from one tube to another by mistake. The gamasids were bred at ordinary laboratory temperature and also in an incubator with a constant temperature of approximately 27° C. The latter, however, though giving optimum conditions for development of *Thrips tabaci*, appeared to be too hot to give good results with the mite.

NOTES ON THE BIOLOGY OF *TYPHLODROMUS THRIPSI* N.SP.

The adult mite is an active creature. There are no eyes, and the first pair of legs appears to be used entirely as tactile organs and not for locomotion. The mite runs rapidly over the leaves of the plant, and when it encounters a thrips larva seizes it at the junction of the thorax and abdomen. The thrips usually lashes its abdomen from side to side in an attempt to shake off the mite, but seldom succeeds if the latter has really gripped the insect. One mite may kill five or six thrips in a day. The young stages, with the exception of the first stage, the larva, are also very active, but the newly hatched mite tends to remain in the place where the egg was laid until after the first moult. It is doubtful if the larval mite feeds.

At laboratory temperatures copulation takes place, on an average, 14 days after the egg has hatched. The shortest time between hatching and copulation was 8 days and the longest 29 days, though it is possible that in the latter instance the mites had already copulated without being observed. Copulation is a prolonged process and may last for some hours. During the process the female runs about the plant in her usual manner with the male attached to her under-side, so that the ventral surfaces are in contact with each other. One female will copulate frequently during her adult life.

At a constant high temperature of 27° C. the interval between hatching and copulation has been as short as 3 days.

Oviposition begins 2–26 days after copulation, but the average time is 12 days. Only one or sometimes two eggs are laid at a time. The female mite lays them at random on the surface of the leaf, with very little attempt to find a sheltered spot, except that as a rule the eggs are found on the under-side of the leaf rather than the upper. After one egg has been laid she usually lays the later ones beside it so that eventually groups of eggs are formed.

The first moult takes place a few hours after the larva has hatched from the egg at 27° C., but at ordinary temperatures it may be delayed as long as 3 days.

DESCRIPTION

Type specimens have been sent to the British Museum.

Typhlodromus thripsi n.sp. *Adult female* (Fig. 1).

Idiosoma: Length approx. ... ... 0·4 mm.
             Width approx. ... ... 0·24 mm.

Legs: 1st pair. Length approx.   ...   0·34 mm.
      2nd pair. Length approx.   ...   0·26 mm.
      3rd pair. Length approx.   ...   0·27 mm.
      4th pair. Length approx.   ...   0·36 mm.

The mite is oval in shape, a little broader at the hind-end and flattened dorso-ventrally. The colour is a light yellowish brown. Under a low magnification the dorsal surface looks quite smooth and polished, but under high magnification a few scattered setae can be seen. The dorsum is almost covered by a plate of chitin, the dorsal shield (Fig. 2). This appears to be single, though a slight constriction about the middle suggests that it may have been formed by the fusion of two plates. There are eighteen pairs of setae, one pair very short, situated in the middle of the anterior border and directed forward over the gnathosoma, one pair, also short, right at the posterior end, and the other sixteen pairs, which are longer and directed backwards, arranged in two series, ten pairs on the anterior and six pairs on the posterior half of the dorsal shield.

On each side below the dorsal plate is a stigma with the peritreme (Fig. 3). The stigma is just posterior to the coxa of the third pair of legs, and the peritreme, which is narrow and slightly undulating, runs forward from it almost to the base of the gnathosoma.

Ventral side. The tritosternum (Fig. 4) is long; the basal piece extends from the anterior border of the sternal plate to the base of the gnathosoma, and the two free ends are approximately the same length as the basal piece.

The sternal plate is nearly square, but the anterior border is convex, with the anterior angles drawn out into points between the coxae of the 1st and 2nd pairs of legs. This plate bears three pairs of setae—one pair at the anterior end, one pair opposite the coxae of the 2nd leg, and the third pair in the posterior angles. The last pair, and the parts of the plate on which they are situated, are separated from the rest of the plate by a faint suture. At the base of the coxae of the 3rd pair of legs are two very small metasternal plates each bearing one seta. The genital plate, which lies in the middle line behind the metasternal plates, bears one pair of setae on the posterior half of the sclerite; the genital opening is on the extreme anterior border and is marked by a thickening of the chitin at this point, and by a peg-like structure projecting forward from it. Under high magnification this peg is seen to be the end of an apodeme, about half of which is inside the body under the genital plate, while the other

half projects forward forming the peg. The genital opening round the peg is fringed with long hairs (Fig. 5). The anal plate is more or less pear-shaped, with the broader end anterior; the anal opening is triangular and occurs near the posterior end of the plate. The anal plate bears four pairs of setae and a single post-anal one. The whole sclerite is traversed by a series of fine striations.

Legs. The 1st and 4th pairs are the longest and are approximately of the same length (0·34 and 0·36 mm. approx.). The 2nd and 3rd pairs are also almost equal in length to each other (0·26 and 0·27 mm. approx.), but the 2nd pair are slightly thicker than the others. The first pair of legs have a larger number of setae than the other three pairs, especially on the distal segments. This is probably due to the fact that the first pair are used exclusively as tactile organs and not for locomotion.

In a preparation of the adult female the "annulated tubes" are conspicuous. These are part of the genital system and appear as a pair of short, thick tubes. One end of the tube is narrow and rounded and appears to have no aperture. There is a tendon attached to the apex of the tube, which is also attached to the synarthodial membrane of the 3rd coxa. At the other end the tube widens out, and in many preparations a round sac is attached to this end of the tube, but this sac is not always visible. In this species the tubes show no signs of annulations of any kind, although by most authors they are described as "annulated tubes" (Fig. 6) (Banks, 1905; Michael, 1892; Vitzthum, 1931).

Gnathosoma (Fig. 7). The mandibles are fairly long and stout, and without hairs. The fixed and movable digits (tibia and tarsus) are equal in length; the fixed digit has a few small teeth.

The coxae of the maxillae form the greater part of the ventral side of the gnathosoma and are divided from each other by a groove, the hypopharynx, running along the midventral line, this groove being partially covered by the arms of the tritosternum. The maxillary palps have the typical five segments and are furnished with hairs which are specially numerous on the two distal segments. The corniculi of the maxillae are well marked, and at their base are three pairs of setae, the setae maxillaris interiores anteriores, the setae maxillaris interiores posteriores and the setae maxillaris exteriores. The maxillary coxae also bear a pair of bristles. There is a very slender sclerite running forward from the hypopharynx between the corniculi of the maxillae.

Adult male (Fig. 9). Only the characters by which the male *Typhlodromus* differs from the female are mentioned.

Idiosoma: Length approx.	...	...	0·30 mm.
Width approx.	...	...	0·17 mm.
Length of legs: 1st pair	...	...	0·28 mm.
2nd pair	...	...	0·23 mm.
3rd pair	...	...	0·23 mm.
4th pair	...	...	0·30 mm.

Ventral side. There is a single sternal-metasternal-genital plate on the ventral side; it is long and narrow and bears five pairs of setae, the same number as is found on the separate plates in the female, placed at the bases of the four pairs of legs and in the posterior angles of the sclerite. The genital opening is at the anterior end, just posterior to the tritosternum, and is shaped like the neck of a flask. The anal plate is broader than that of the female, and while it also has four pairs of setae and a post-anal one, their arrangement is different (Trägårdh, 1911).

Legs. The 4th pair of legs is equal in length to the idiosoma and the 1st pair is nearly as long; the two middle pairs are shorter.

Gnathosoma (Fig. 8). The mouth-parts are similar to those of the female, except that the mandibles have a "spermatophore carrier". This is a spine arising from the outer side of the movable digit of the mandible. The spine projects forward in front of the mandible and its free end is forked. It serves to convey the spermatophores from the male genital opening and insert them into that of the female (Michael, 1892; Oudemans, 1915).

The egg. The egg is an oval, translucent body, approximately 0·2 mm. in length by 0·12 mm. at its broader end. The variation in its width is slight. It is colourless and without sculpturing.

Larva (Fig. 10). The egg hatches into a six-legged larva. This is completely colourless and is not so flattened dorso-ventrally as the later stages. Except that there are only three pairs of legs, the general form does not differ greatly from the adult, but the arrangement of the plates on the dorsal and ventral sides is different. The dorsal plate is divided by a suture into two unequal parts; the anterior part is larger and bears nine pairs of setae; there is one pair at the anterior edge of the plate, directed forwards as in the adult, but most of the setae are longer and stouter in proportion than in the adult, and a particularly long, strong pair projects laterally between the 2nd and 3rd pairs of legs. The posterior part of the dorsal plate has only two pairs of bristles, a short pair near the anterior margin, and a very long, curved pair on the extreme posterior border (Fig. 11).

On the ventral side there are two plates (the anterior one resembles the sternal-metasternal genital plate of the adult male, but has only three pairs of setae) and the anal shield, also similar in shape to that of the adult male, but bearing six pairs of setae as well as the post-anal one.

The mandibles (Fig. 12) resemble those of the female mite; a spermatophore carrier is never present.

No trace of the stigma or the peritreme could be found.

Protonymph (Fig. 13). After the first moult the mite has four pairs of legs and is known as a protonymph.

The dorsal plate (Fig. 14) still shows traces of a division; the anterior half bears eleven pairs of setae, and the posterior eight pairs. The pair of setae right at the posterior end of the plate are very much shorter than in the larva.

Only two plates can be distinguished on the ventral side, and these are similar to the ventral plates in the preceding stage, except that on the anal plate the setae are reduced in number to four pairs and a single post-anal seta, as in the adult mite. The stigma and the peritreme can be seen in this stage, the stigma situated, as in the adult, just anterior to the fourth coxa, but the peritreme (Fig. 15) is very short, only reaching to the base of the third pair of legs.

The mandibles are like those of the larva.

Deutonymph. In this stage the anterior plate on the ventral side is almost the same as in the adult male, and bears five pairs of setae, but there is no genital aperture. The peritreme is longer than in the protonymph but not so long as in the adult. The mandibles are similar to those of the adult female.

SUMMARY

1. *Typhlodromus thripsi* n.sp., of which a description is given, is a predacious mite feeding on *Thrips tabaci* Lind. It appears to be one of the few natural enemies of this insect.

2. There are five stages in the life cycle of the mite; these are: egg, larva, protonymph, deutonymph and adult.

3. The mites first appear in June and are found on the plants until December, by which time the thrips have disappeared for the season.
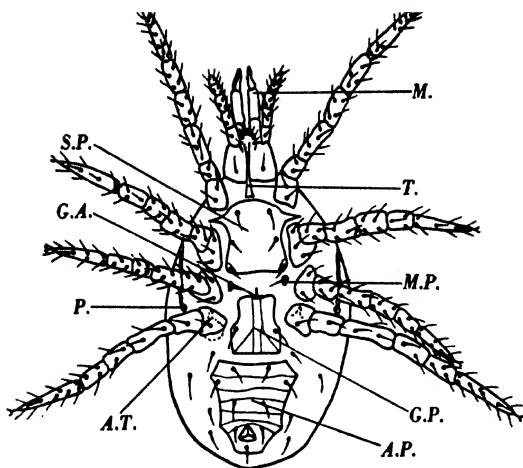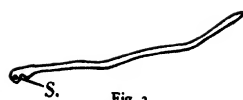
Fig. 1

Fig. 3
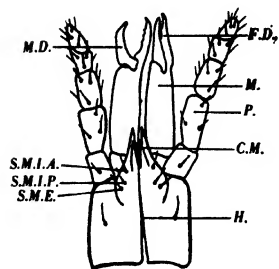
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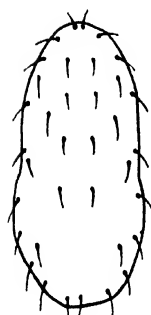
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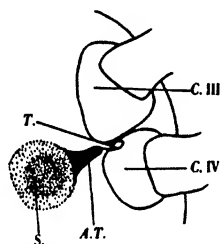
Fig 6

Fig 4

Fig. 5

Fig. 8

Figs. 1–9.

Fig 9

S.P.

A.P.

Fig. 10

Fig. 11

Fig 12

S.
Fig. 15

S.P.

P.

A.P.
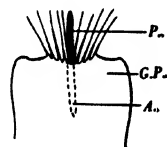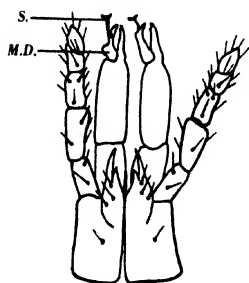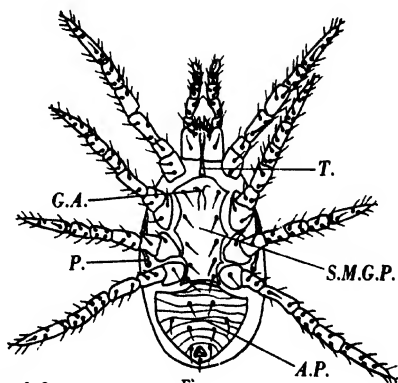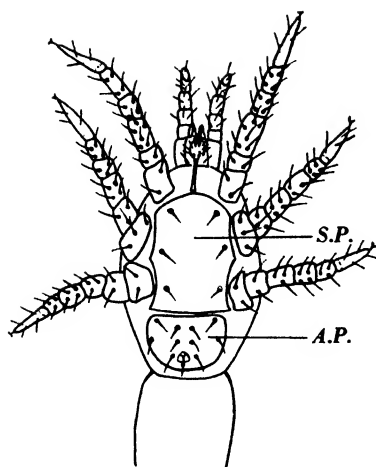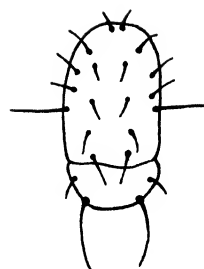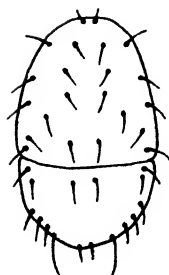
Fig. 13

Fig. 14
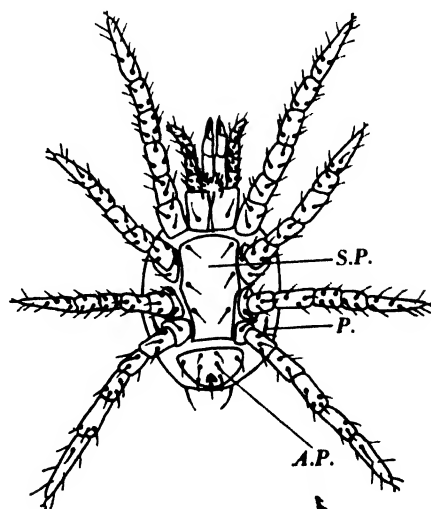
Figs. 10–15.

REFERENCES

BANKS, N. (1905). A treatise on the *Acarina* or mites. *Proc. U.S. nat. Mus.* **28**, 1.

MICHAEL, A. D. (1892). On the variations in the internal anatomy of the Gamasinae. *Trans. Linn. Soc. Lond.* (*Zool.*), **5**, pt 9, 281.

OUDEMANS, A. C. (1915). Notizen über Acari. *Arch. Naturgesch.* **81**, 154.

—— (1929–33). Acarologische Aanteekeningen. XCLX. *Ent. Ber., Amst.*, **8**, 14.

SCHEUTEN, A. (1857). Einiges über Milben. *Arch. Naturgesch.* **23**, 104.

TRÄGÅRDH, I. (1911). Contributions towards the comparative morphology and phylogeny of the Parasitidae (Gamasidae). *Ark. Zool.* **7**, no. 28, 1.

VITZTHUM, H. Graf (1931). Acari. *Handbuch der Zoologie*, p. 44. Berlin and Leipzig: Kükenthal.

EXPLANATION OF FIGS. 1-9.

Fig. 1. Adult female. Ventral side. × 100. *A.P.* anal plate; *A.T.* position of "annulated tube"; *G.A.* genital aperture; *G.P.* genital plate; *M.* mandible; *M.P.* metasternal plate; *P.* peritreme; *S.P.* sternal plate; *T.* tritosternum.

Fig. 2. Dorsal shield. Adult. × 110.

Fig. 3. Peritreme. Adult. *S.* stigma. × 240.

Fig. 4. Tritosternum. × 330.

Fig. 5. Genital aperture. Female. × 740. *A.* part of apodeme under genital plate; *G.P.* genital plate; *P.* "peg"—free part of apodeme.

Fig. 6. "Annulated tube." × 370. *A.T.* "annulated tube"; *C.* III. 3rd coxa; C. IV. 4th coxa; *S.* sac; *T.* tendon.

Fig. 7. Gnathosoma. Adult female. × 220. *C.M.* corniculus of maxilla; *F.D.* fixed digit of mandible; *H.* hypopharynx; *M.* mandible; *M.D.* movable digit of mandible; *P.* maxillary palp; *S.M.I.A.* seta maxillaris interiores anteriores; *S.M.I.P.* seta maxillaris interiores posteriores; *S.M.E.* seta maxillaris exteriores.

Fig. 8. Gnathosoma. Adult male. × 250. *M.D.* movable digit of mandible; *S.* spermatophore carrier.

Fig. 9. Adult male. Ventral side. × 100. *A.P.* anal plate; *G.A.* genital aperture; *P.* peritreme; *T.* tritosternum; *S.M.G.P.* sternal-metasternal-genital plate.

EXPLANATION OF FIGURES 10-15.

Fig. 10. Larva. Ventral side. × 110. *A.P.* anal plate; *S.P.* sternal plate.

Fig. 11. Dorsal plate. Larva. × 110.

Fig. 12. Mandible. Larva. × 740.

Fig. 13. Protonymph. Ventral side. × 100. *A.P.* anal plate; *P.* peritreme; *S.P.* sternal plate.

Fig. 14. Dorsal plate. Protonymph. × 110.

Fig. 15. Peritreme. Protonymph. × 550. *S.* stigma.

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SOME GALL MIDGE SPECIES AND THEIR HOST PLANT RANGE

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(With Plate XXII)

CONTENTS

I. INTRODUCTION

BROADLY speaking the taxonomist may take up one of two attitudes: either every insect sent in for identification can be considered as possibly new and undescribed until it is proved to be the same as one previously described, or else it can be presumed that the insect in question has already been described and must not be considered new until it can be proved to be different from all those previously described.

The latter method results in a tendency to classify temporarily as one several distinct species. From a biologist's point of view this may subsequently be disastrous if it is later shown that two or three species are really involved. All the previous literature concerning the bionomics and control in such a case becomes comparatively worthless and new investigations have to be commenced on each of the species.

The former method appears to be the more logical when it is realized that the number of known species is often but a fraction of those awaiting discovery, and that in many groups the present classification must of necessity be considered merely as the most convenient for the moment.

In the Cecidomyidae or gall midges, it is well known that it is sometimes exceedingly difficult to differentiate between closely allied species. This being so, workers in this group have been inclined to look upon midges reared from different host plants as separate species and then,

acting on this presumption, to find some minute differences of a morphological nature on which to base the description of the new midge. There is some excuse for this method. For instance, when only a few specimens are available, such minute differences appear real. (A vastly different state of affairs exists when one is dealing with hundreds of either sex, as in biological studies.) An advantage is that it keeps possibly distinct species apart until such time as it can be proved that they are identical. A name, apart from taxonomic considerations, is also an identification tag or key whereby it should be possible to look up all the available information concerning the insect in question.

However, as is illustrated below, this is precisely what so often does not result if each worker uses a different name (and therefore a different tag) for what in reality is one species. From an economic entomologist's point of view such a procedure can be very misleading, particularly if there is no subsequent literature concerning many of the species.

For example, in the literature concerning the species of the genus *Asphondylia*, 133 species have been described with host plant records. The plants recorded occur in forty families. All the 133 species are each limited to one family of host plants.[1] Among the *Asphondylia* species, 127 are limited to one genus of host plant, five species to two genera and one to three genera. Again, 103 species are limited to one species of host plant, fourteen to two species, four to three species, six to four species, three to five species, two to six species and one to twelve species; and in only a few instances has anything been published concerning these species since the original description and host plant record. If the question arose whether it would be considered a safe measure to introduce a certain *Asphondylia* species as a weed control, would there be any justification in the present state of knowledge in postulating that it was unlikely that the particular midge in question would limit its depredation to the only known host plant species?

Again, in the older literature there are references to *Contarinia medicaginis* Kieffer attacking the unopened blossom of *Medicago* spp. and *Lotus corniculatus*, and to *Contarinia loti* D.G. doing the same damage to *Lotus* spp. and also *Medicago* spp. and *Vicia* spp. These two species are comparatively common pests of seed production and numerous references to them occur in the literature. Yet it is only recently that the view that *Contarinia medicaginis* limits its attention to species of

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[1] The only recorded instance of a gall midge living on plants belonging to two families is that of *Macrolabis corrugans* F.Lw. which is reported to live on certain Umbelliferous and Labiate species.

Medicago, and *Contarinia loti* confines itself to species of *Lotus*, has been accepted among those most intimately concerned.

The differences recorded in the descriptions of midge species are often so slight that it is wellnigh impossible even if the original specimens are examined to be sure that such differences are really specific and valid. The range of variation of such differences within a species is, with very few exceptions, almost unknown (Barnes, 1932).

Again, as, for example, *Rhabdophaga triandraperda* Barnes, *R. purpureaperda* Barnes and *R. justini* Barnes, some species are exceedingly difficult to separate on the adults alone. But the pupae, larvae and host plants taken together serve as a ready means of identification (Barnes, 1935 *a*). Among the species of the genus *Contarinia* living in grass-heads there appear few, if any, distinguishing morphological characters among the adults, pupae and larvae, and one has to depend on a knowledge of the host plants. This can only be obtained by biological experimentation.

It is evident therefore that what is needed, both for the grower and taxonomist, is that far more attention should be paid to acquiring biological information (for example, host plant range) and the emphasis taken away from the use of pure morphology alone as a means of separating species. The grower wants answers to such questions as: If he suffers from *C. loti* on his *Lotus*, can he safely grow Lucerne? If his *Salix triandra* is attacked by *Rhabdophaga triandraperda*, can he expect his *Salix purpurea* and *S. viminalis* to escape damage?

The present series of studies is based on such a motif. In the first instance the *Arabis* midge will receive consideration. Then will follow accounts of similar investigations on the chrysanthemum midge, the black-currant leaf midge and the hawthorn stem midge.

II. THE *ARABIS* MIDGE

Historical

In the literature there appear three names of midges whose larvae cause terminal bud galls on *Arabis* species, namely, *Dasyneura alpestris* Kieffer (1909), *D. schneideri* Rübsaamen (1917) and *D. arabis* Barnes (Barnes & Theobald, 1927). A brief survey of the published information concerning these midges follows:

Dasyneura alpestris Kieffer. In 1909 Kieffer wrote:

"*Arabis alpina* L. et *hirsuta* Sc. Pousse terminale changée en une agglomération de feuilles élargies (Thomas, 1886). *Perrisia?* *alpestris* sp.n."

In this way Kieffer gave a name to the insect whose larvae had previously been noticed causing a terminal bud gall on *Arabis alpina* at Suldenthal in the Tyrol by Thomas (1886).

F. Loew (1888) mentioned a similar gall on *A. hirsuta* which he had found in Niederösterreich. Schlechtendal (1891), in his list of German gall formers, noted a midge doing this type of damage to *A. alpina* and *A. hirsuta*. Rübsaamen (1896), in his paper on Russian gall midges, stated that the midge causing the gall described by Thomas (1886) belonged to the genus *Dichelomyia*, but did not actually say that it had been found in Russia. de Meijere (1928) described the adults of a midge which he had found in Holland in terminal bud galls of *A. alpina* and retained the name *alpestris* Kieffer, calling the midge *Dasyneura alpestris* Kieffer. de Meijere at the same time suggested that this species was synonymous with *D. schneideri* Rübsaamen.

Dasyneura schneideri Rübsaamen. Rübsaamen (1917) described in detail, as *D. schneideri*, a midge whose larvae caused terminal bud galls on *Arabis albida* Stev.[1] At the same time he mentioned finding white larvae with "breastbones" slightly differing in shape from those of *Dasyneura schneideri* whose larvae were red. The adult midges belonging to this second species were not known to Rübsaamen who received *D. schneideri* from Dr Schneider-Orelli from Zurich.

Later the Deshusses (1936) reported *D. schneideri* from Switzerland doing extensive damage on *Arabis albida*. They stated that they had reared platygasterid parasites and gave information concerning the biology, and a description of the adult midge. There are excellent photographs of the gall in this paper. In addition, they mention that similar damage had been recorded on *A. alpina* in Switzerland by Moreillon (1916), Vogler (1906) and Perriraz (1909). Lastly these authors state that a memoir on the anatomy of *Dasyneura schneideri* will appear later.

Dasyneura arabis Barnes. In 1927 the present writer (Barnes & Theobald, 1927) described, as *D. arabis*, midges reared in England from larvae which answered the description of the second midge mentioned by Rübsaamen (1917), but whose adults were then unknown. Later Barnes (1935 b), in a study of fluctuations in insect populations dealing with *D. arabis*, wrote: "It seems quite possible that in the future it will be proved that these two species referring to *D. schneideri* and *D. arabis* are identical and that the differences observed between them fall within the range of variation." And later in the same paper he stated that *Arabis alpina* and *A. hirsuta* had not yet been tested as possible host plants of *Dasyneura arabis*. A species of *Lestodiplosis* was reported at that time as being predaceous on the larvae of *Dasyneura arabis* and *Misocyclops marchali* Kieffer was recorded as an internal parasite.

Present investigations

In the autumn of 1937 Dr H. Sachtleben gave the writer the opportunity of examining midges reared by Dr Pape, who had found the galls on *Arabis alpina* at Bad Soden-Allendorf and Kiel. These specimens answered exactly the description of *Dasyneura alpestris* Kieffer given by de Meijere in 1928.

At the same time inquiries revealed that *D. arabis* was present in

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[1] This species is now considered a synonym of *Arabis caucasica* Willd.

England in Surrey, Middlesex, Hertfordshire and Cambridge. On the other hand, searches made for the galls were unproductive in Manchester, Oxford, Newcastle, Cornwall, Somerset, Devon and Caernarvonshire. Incidentally, in 1937 wherever the midge occurred it was found in great abundance. For instance, in almost every garden where *Arabis caucasica* was growing in Harpenden the galls were exceedingly obvious in September. On the other hand, in 1938 the galls were not at all plentiful.

The opportunity had arisen for attempting to determine biologically the status of the three species, *Dasyneura alpestris*, *D. schneideri* and *D. arabis*.

Accordingly, in September 1937, living material was obtained on *Arabis alpina* from Kiel, Germany and the Royal Botanic Gardens, Kew; on *A. alpina* var. *rosabella* from Kew; and on *A. caucasica* (*albida*) from the garden of Rothamsted Experimental Station, Harpenden, from Batford, Hertfordshire, and from Cambridge. Adult midges emerged in the outdoor insectary from the English material between 6 and 29 October and from the German material from 17 October to 18 November. These dates are much later than the latest previously[1] known (11 October) for *Dasyneura arabis* and probably partly account for the comparative scarceness of the midge in 1938. The larvae normally overwinter in the soil and in their cocoons on the plants. If most of the third, as in this case, or probably the fourth generation emerge and the resultant larvae cannot reach the necessary stage of development before adverse conditions set in, then there will be comparatively few individuals overwintering successfully. This has been observed previously in the leaf-curling pear midge (Barnes, 1935 c).

In addition to the extremely late emergences in October and November 1937, the midges started emerging in 1938 much sooner than in any year between 1929 and 1934. In the exceptionally early season of 1933 the first emergence occurred on 17 April instead of between 4 and 17 May in the other years. But in 1938 emergence started on 6 April. Frosty weather subsequently followed, which was another factor in decreasing the amount of damage due to this midge in 1938.

During the rearing of the *Dasyneura* midge material in 1938, besides breeding a few specimens of the *Lestodiplosis* sp. which had been previously reared, specimens of another midge were also bred. They belong to the genus *Macrolabis*. The breeding of this *Macrolabis* species probably provides the clue to the white larvae mentioned by Rübsaamen

[1] For details of the biology see Barnes (1935 *b*).

(1917) and their supposedly adult midges described by the present writer as *Dasyneura arabis* in 1927. For it was found that when dealing with large numbers of larvae in the fluctuation study (1935 *b*) the colour of the gall-forming larvae varied from white to red, and the suspicion was then aroused as to the validity of the separation of *D. arabis* from *D. schneideri*. Parasites were also bred from the English and German material and have been identified by Dr Ferrière as follows: Eulophidae, *Tetrastichus annulatus* Först. (Kiel on *Arabis alpina*, Kew on *A. alpina* var. *rosabella* and *A. alpina* and Harpenden on *A. caucasica*); *Omphale aetius* Walk. (Harpenden on *Arabis caucasica*). Platygasteridae, *Synopeas* sp.n.? (Kew on *Arabis alpina* and its variety *rosabella*); *Piestopleura catillus* Walk. (Kew on *Arabis alpina*).

Cross-mating experiments, 1938

In April 1938 newly emerged midges, chosen for their difference in previous host plant and locality, were given the opportunity of mating in small glass tubes standing in sand. All the crosses were successful, mating taking place in the normal time and lasting the usual period (Table I).

Table I. *Cross matings of* Arabis *midges reared on different* Arabis *spp. and from different localities*

	Male		Female	
	Sp. of *Arabis*	Source	Sp. of *Arabis*	Source
1.	*A. alpina* var. *rosabella*	Kew, Surrey	*A. alpina*	Kiel, Germany
2.	*A. alpina*	Kew, Surrey	*A. caucasica*	Batford, Herts.
3.	*A. alpina*	Kew, Surrey	*A. caucasica*	Cambridge
4.	*A. alpina*	Kew, Surrey	*A. caucasica*	Cambridge
5.	*A. alpina*	Kew, Surrey	*A. alpina*	Kiel, Germany
6.	*A. alpina*	Kew, Surrey	*A. alpina*	Kiel, Germany
7.	*A. caucasica*	Batford, Herts.	*A. alpina*	Kew, Surrey

In every case followed up, the cross matings were fertile and, in order to test the interchangeability of the host plant species, the females were allowed to oviposit on different species of *Arabis* (Table II).

Table II. *Breeding of the cross-mated midges on various* Arabis *spp.*

Cross mating					
Male		Female			
Host plant sp.	Locality	Host plant sp.	Locality	Sp. of *Arabis*	Fertility
1. *A. alpina*	Kew	*A. caucasica*	Batford	*A. aubrietioides*	Normal
2. *A. alpina*	Kew	*A. caucasica*	Cambridge	*A. caucasica*	Normal
3. *A. caucasica*	Batford	*A. alpina*	Kew	*A. alpina*	Normal
4. *A. alpina* var. *rosabella*	Kew	*A. alpina*	Kiel	*A. caucasica*	Normal

It is of interest that in two cases where only one female was used, the offspring or G_1 showed a marked departure from the normal 50 : 50 sex ratio, thus supporting the previous finding that in *Dasyneura arabis* there are male-producing females and female-producing females in addition to females which produce families in which both sexes occur in approximately equal numbers (Barnes, 1931 *a*).

Natural infestation of Arabis *spp.*

In late September 1937 the *Arabis* spp. growing in the Royal Botanic Gardens, Kew, were inspected and it was noted that while some species were heavily infested, others near by had suffered no attack. It was evident that all the species of *Arabis* which looked like *A. caucasica* were attacked while other species, rather different in general appearance, showed no signs of damage or galls. Table III shows the species examined at Kew, those attacked by the midge, and the subgenus to which they belong. The writer is indebted to the courtesy of the Director and the authorities of the Herbarium, Royal Botanic Gardens, Kew, for the correct citations, names of the subgenera and the systematic notes concerning the species of *Arabis*. It can be seen from Table III that some species of the subgenus *Euarabis* were attacked by the midge (but not all the species of *Euarabis* which were present). and one species which may belong to the subgenus *Lomaspora*.

Immunity trials

Having observed a definite preference of the midges for certain species of *Arabis*, it was decided to subject the midges to immunity trials, i.e. give them the obligation to oviposit on a species of *Arabis* or else produce no offspring.

Accordingly, midges were introduced into cages containing only one of the following *Arabis* spp., obtained through the courtesy of the Director of the Royal Botanic Gardens, Kew: *A. alpina, A. aubrietioides, A. caucasica, A. carduchorum, A. hirsuta* var. *glabra, A. landaurii, A. procurrens* and *A. Scopoliana*. Five or six freshly emerged and impregnated females were placed in each cage in the trials carried out in April 1938, two pots of each species of *Arabis* being used, with the exception of *A. hirsuta* var. *glabra*, where only one plant was available. In the August trials three females of the midge were used in each cage and two plants of *A. Scopoliana* and *A. procurrens*, and one plant each of *A. landaurii, A. hirsuta* var. *glabra*, and *A. carduchorum* were subjected to trial.

After the insertion of the midges into the cages, observations were

made for the first few hours for signs of oviposition. In the *A. alpina*, *A. aubrietioides* and *A. caucasica* pots, oviposition was observed without exception to take place almost immediately. With all the other *Arabis*

Table III. *Arabis spp. at the Royal Botanic Gardens, Kew, indicating which were suffering from midge attack* on 28 September 1937

Arabis spp.	Subgenus	Midge galls present
A. alpina L.	*Euarabis*	×
A. alpina L. var. *rosabella*[1]	*Euarabis*	×
A. androsacea Fenzl.	*Pseudarabis*	–
A. aubrietioides Boiss.	*Euarabis*	×
A. bellidifolia Jacq.	*Euarabis*	–
A. blepharophylla Hook. & Arn.	*Euarabis*	–
A. brachycarpa Rupr.	*Lomaspora*	–
A. Breweri S.Wats.	*Turritis*	–
A. carduchorum Boiss.	*Drabopsis*	–
A. caucasica Willd. (*albida* Stev.)	*Euarabis*	⁄
A. Collinsii Fernald	*Turritis*	–
A. corymbiflora Vest.	*Turritella*	–
A. hirsuta Scop. var. *glabra* L. (Lange)[2]	*Turritella*	–
A. japonica Regel	See footnote [3]	–
A. Koehleri[4]	*Turritis*	–
A. landaurii[5]	*A. bellidifolia* × *Ferdinandi Coburgi*	–
A. Lyallii S.Wats.	*Turritis*	–
A. procurrens Waldst. & Kit.	*Pseudarabis*	–
A. Scopoliana Boiss.	*Drabina*	–
A. Stelleri DC. var. *rosea*	*Lomaspora?*[6]	×
A. tomentosa[7]	See footnote [7]	–

[1] ? horticultural form of *A. alpina*.

[2] *A. hirsuta* Scop. var. *glabra* L. (Lange) in *Fl. Dan.* 17, t 2911, where the name *Turritis hirsuta* var. *glabra* L. is quoted in synonymy.

[3] *A. japonica* Regel et Herder (1863) (which may or may not be the same as *A. japonica* (A. Gray) A. Gray, 1858) was referred by its authors (*Gartenfl.* 12, 309) to Sect. *Alomatium* DC. But they also stated that it stood "about midway between *A. alpina* L. and *A. brassiciformis* Wallr.", which are referred by O. E. Schulz (*Engl. Pflanzenfam.* ed. 2, 17, 544, 1936) to Sect. *Brassicoturritis* O. E. Schulz. *A. japonica* (A. Gray) A. Gray is treated by Nakai (in *Bot. Mag. Tokio*, 32, 241, 1918) as a var. *japonica* (A. Gray) Fr. Schmidt of *A. Stelleri* DC., a species placed by O. E. Schulz (*Engl. Pflanzenfam.* ed. 2, 17, 545, 1936) in Sect. *Turritella* C. A. Mey.

On the basis of the original description, *A. japonica* Regel et Herder runs down in Schulz's key (*Engl. Pflanzenfam.* ed. 2, 17, 543, 1936) to Sect. *Brassicoturritis*, but the sectional differences, depending on the erect or ascending position of the fruits and/or pedicels, seem very slight.

[4] Name not traced at Kew.

[5] A letter from the Director, Royal Botanic Gardens, includes the following statement: "*Arabis Landaueri* Sundermann in *Allg. Bot. Zeitschr.* 26–27, 21 (1925), hybr.-Hort. We think this is what is meant by your name '*A. landaurii*'."

[6] *Stelleri* DC. is described in DC. *Syst.* 2, 242 as most closely allied to *pendula*, which belongs to Sect. *Lomaspora*. A subsequent letter from the Director, Royal Botanic Gardens, includes the statement: "We cannot trace *Arabis Stelleri* var. *rosea*; we find, however, *A. Stelleri* DC., *Syst.* 2, 242 (1821), and also *A. rosea* DC., *Syst.* 2, 215."

[7] Name not traced at Kew.

spp., both in April and in August, attempts at oviposition, still less actual oviposition, were never observed.

Later, the plants were examined for galls. In each pot of *A. alpina*, *A. aubrietioides* and *A. caucasica* galls which ultimately gave rise to adult midges were observed. On the other hand, no galls and consequently no adult midges appeared on *A. carduchorum*, *A. hirsuta* var. *glabra*, *A. landaurii*, *A. procurrens* and *A. Scopoliana*.

Conclusions

In the light of these biological tests supported by the knowledge of the midges themselves, one is forced to the conclusion that there is only one real species involved in the formation of terminal leaf-bud galls on *Arabis*.

This species should be known as *Dasyneura alpestris* (Kieffer, 1909) de Meijere (1928), the adults of which were first described by de Meijere in 1928. The gall described by Kieffer is quite easily recognizable from the description and this type of gall is not caused on these plants by any other gall midge. The names *Dasyneura schneideri* Rübsaamen (1917) and *D. arabis* Barnes (1927) fall as synonyms.

D. alpestris (Kieffer) de Meijere will only live on certain *Arabis* spp. belonging to the subgenus *Euarabis* and possibly also *Lomaspora*, and will not attack species belonging to such other subgenera as *Pseudarabis*, *Turritis*, *Drabopsis*, *Drabina* and *Turritella*. The midge will not, however, attack all species of the subgenus *Euarabis*. It is shown to develop normally on *Arabis alpina*, *A. alpina* var. *rosabella*, *A. aubrietioides* and *A. caucasica*. Attempts to induce it to breed on five species in other subgenera failed.

In the literature the midge is recorded from *A. hirsuta*. This species itself has not been used in the experiments but its variety, *A. hirsuta* var. *glabra*, proved to be unattacked by the midge.

The second midge mentioned by Rübsaamen (1917) is probably the *Macrolabis* species which has been found living, and reared, as a commensal in the galls of *Dasyneura alpestris*.

III. The chrysanthemum midge

Briefly stated, the question to be considered is whether the chrysanthemum midge of commercial varieties is the same species as *Diarthronomyia hypogaea* F.Lw. of wild *Chrysanthemum* spp. It is important to note that in all the experiments described subsequently only midges

obtained from cultivated chrysanthemums have been used. No specimens from wild host plants have been available.

Distribution of the commercial chrysanthemum midge

United States of America. Felt (1915) identified as *Diarthronomyia* (*Rhopalomyia*) *hypogaea* F.Lw. (see next section) a midge which was causing serious damage to commercial chrysanthemums in a glasshouse during late March of that year at Adrian, Michigan. This is the first record of the chrysanthemum midge attacking commercial varieties, although according to Essig (1916) Californian florists stated that this pest had been present round San Francisco Bay for over 15 years. In 1915 it was definitely recorded from California and Oregon as well as from Michigan (Felt, 1916). By 1920 the midge had been reported from all the larger chrysanthemum-growing regions of the U.S.A., including California, Connecticut, Delaware, the District of Columbia, Georgia, Illinois, Indiana, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Ohio, Oregon, Pennsylvania, Rhode Island, South Dakota, Tennessee and Virginia (Weigel & Sanford, 1920).

Canada. This midge first appeared in Canada in 1915 at Ottawa where it occurred in one of the large greenhouses and, according to Gibson (1916), had doubtless been recently introduced with plants from the U.S.A. In August of the same year it was found on commercial chrysanthemums growing both outside and inside a greenhouse in Victoria, B.C. (Gibson, 1917; Treherne, 1916). Since that date it has become well established throughout the province of Ontario and has also been found in Quebec and Nova Scotia (Gibson & Ross, 1922).

Russia. Possibly the first European record of this midge on commercial varieties of chrysanthemum is that by Daniltchenko (1916). This writer, when dealing with chrysanthemums and their cultivation, stated that the collar is injured by this pest. Pending further information this record can only be considered as a warning to chrysanthemum growers. No exact information as to its status in Russia is available, but Miss Dombrowski has informed the writer (*in litt.* March 1937) that she has found *Diarthronomyia hypogaea* only on wild *Matricaria inodora* L. and *M. chamomilla* L.

England. The first outbreak was discovered in England in 1927 in the Lea Valley (Speyer, 1927 *a*, *b*, 1928) and two subsidiary infestations were noted in Bedfordshire and in Norfolk (M. of A. 1928). In the autumn of 1928 (Miles, 1929) the midge was found in a commercial glasshouse in South Lancashire. Two infestations were known to persist in the country in the autumn of 1930 (M. of A. 1933), but in 1936 (M. of A. 1936) the Ministry of Agriculture was in a position to state that the eradication campaign had been successful as no occurrence of the pest had been found since 1930.

The second outbreak in England occurred in 1936 and the midge was discovered in several nurseries in Sussex, with four isolated infestations in Essex, Gloucestershire and Glamorganshire.

Up to December 1938 the midge was still present in Sussex and Essex.

Both these outbreaks were traced to chrysanthemum varieties imported from the U.S.A.

Ireland. Mr R. Chamberlain has recently informed the writer that the midge was found on 31 December 1937 at Belfast. In this case the outbreak was traced to an infested area in England.

Denmark. The midge was first recorded attacking commercial chrysanthemums in Denmark in 1934. P. Bovien (1935) found galls in the open and in greenhouses during the winter. The same writer (1937) reported its occurrence in 1936 in two new localities including the island of Funen near Copenhagen.

Finland. The midge first appeared in 1936 in Finland and has become established in a few glasshouses in the vicinity of Helsinki and Turku. In one case it was evidently imported from Germany and in another from Sweden. The writer is indebted to Mr Niilo A. Vappula for this information and to Mr Y. Hukkinen for specimens.

Sweden. Commercial chrysanthemums were found infested by this midge in the Stockholm area early in November 1936 (Statens Växtskyddsanstalt, 1936) and it is stated that probably it was imported from Denmark. The midge was found in at least twelve places from Skåne northwards to Southern Norrland, and in two instances it had been seen by growers in 1935 but not reported. Later, Ahlberg (1938) stated that irregular watering and high humidity are partly responsible for the depressions found near the actual galls.

France. Suire (1935) mentions *Diarthronomyia hypogaea* Lw. in his list of insects attacking chrysanthemums in France. Prof. A. Balachowsky (*in litt.* 10 October 1938) stated that nothing has been published recently in France concerning this midge attacking autumn chrysanthemums.

Germany. Dr Hans Sachtleben of the Deutsches Entomologisches Institut has informed the writer (*in litt.* 8 September 1938) that the occurrence of the midge on cultivated chrysanthemums in Germany was till then slight and only local.

From the above summary of the midge's distribution on commercial chrysanthemums, it can be seen that it first occurred as a pest in the United States of America. By 1916, only one year after it was first recorded, besides being established in California, Oregon and Michigan, it had already been reported from British Columbia and Ottawa in Canada. The first definite occurrence of the midge as a pest in Europe was in England in 1927, and a further outbreak began in 1936. Other outbreaks have occurred in Europe in recent years. It appeared in Denmark first in 1934 and apparently is still present. In 1935 it arrived in Sweden and in the next year reached Finland.

Identification

When the chrysanthemum midge was first discovered in the United States of America, Felt (1915) identified it as *Diarthronomyia* (*Rhopalomyia*) *hypogaea* F.Lw.

This species had been described by F. Loew (1885) from specimens of subterranean galls, two males and pupae found on *Chrysanthemum atratum* Jacq. in Central Europe (Raxalpa) by E. Berroyer in 1875. Lemée (1902) recorded this midge forming galls on the flowers, leaf buds and stems of *C. Leucanthemum* L. in France, and Baldrati (1900) reported and figured it from the same plant in Italy. Lemée (1902) also recorded

it from *C. corymbosum* L. and a midge, possibly the same species, on *C. japonicum* Thunb.[1] Cotte (1912) recorded it from *C. Myconis* L. and figured the gall (Houard, 1913).

During recent years doubts have been growing as to whether the midge of commercial chrysanthemums is *Diarthronomyia hypogaea* F.Lw. (1885). The galls figured by Baldrati and by Cotte bear little resemblance to those caused by the midge on commercial chrysanthemums and, moreover, the commercial chrysanthemum midge has not been known to spread to wild *Chrysanthemum* spp. For example, Weigel & Sanford (1920) wrote: "Although several attempts have been made to infest the Shasta daisy and the common field daisy, *C. Leucanthemum*, it has not been possible to get the ovipositing female to lay eggs on them." Again, Hamilton (1924) stated that experiments were carried out to see if the midge would reproduce on any of the chrysanthemums other than the cultivated greenhouse varieties. *C. Leucanthemum* and its cultivated variety the Shasta daisy (*C. maximum*) were used but the females did not oviposit on them. An infestation was secured on the annual or summer varieties (mixed seed and probably *C. coronarium*). Eggs were laid on four of the plants and galls subsequently formed on all four. Distinct galls were not formed but the leaves were curled and twisted while the stems and buds were deformed. No adult midges however developed.

Similarly, Miles (1929) failed to find signs of its presence on wild ox-eye daisy in various parts of south Lancashire and north Cheshire, although, in the autumn of 1928, the chrysanthemum midge was occurring on commercial plants in south Lancashire. Efforts on the part of the writer to find the midge on wild ox-eye daisy from 1927 to date have also failed. Mr N. A. Vappula (*in litt.* 1 September 1938), in reply to the question whether it had been found on wild chrysanthemums in Finland, wrote that up to the present it had been found only on glasshouse chrysanthemums. Similarly Mr P. Bovien (*in litt.* 23 July 1938) stated that he had not found it on *C. Leucanthemum* in Denmark.

The following quotation from an unsigned article in the *Journal of the Ministry of Agriculture* (Anon. 1937) summarizes the current opinion held in England: "When the midge (*Diarthronomyia* sp.) was first discovered in America it was recorded under the name of a European species (*Diarthronomyia hypogaea* F.Lw.), known since 1885 as attacking the roots of various kinds of ox-eye daisy. This original identification, however, seems to have been accepted in Europe without further investigation and without experimental evidence to show whether the daisy

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[1] In *Index Kewensis* this species is considered to be *C. indicum* L.

midge will in fact attack greenhouse chrysanthemums. In view of the difference in habit between the two insects, it is not improbable that they may prove to be distinct species. However this may be, it is clear from the horticultural point of view the chrysanthemum midge must be regarded as a foreign pest...."

Present investigations

Fruitless efforts have been made to rediscover *Diarthronomyia hypogaea* F.Lw., reported by Bagnall & Harrison (1921) on *Chrysanthemum Leucanthemum* at Babbacombe and Sidmouth, Devon, both in the latter locality and elsewhere in England. Similarly, attempts to obtain material from wild chrysanthemums on the continent of Europe have proved of no avail.

Material of the chrysanthemum midge on commercial chrysanthemums, however, has been supplied by Mr A. S. Buckhurst of the Ministry of Agriculture from the Worthing area in February 1937 and from south Wales in December 1937.[1]

The Ministry of Agriculture having granted the writer a licence to keep specimens of the chrysanthemum midge, an attempt has been made to find the host range of this midge and also to study its life history in this country. A later report will deal with the latter. The following section gives the results of attempts to induce the midge to breed on different species of *Chrysanthemum* and other allied plants.

Host range

(i) *Species and varieties of* Chrysanthemum.

Commercial varieties of autumn chrysanthemum. The following varieties were used for breeding the midge both in the laboratory and in an unheated open greenhouse: Crimson Circle, September White, September Pink, Phoenix, Mrs Arkwright, "Rose Pink" and Cranford Yellow. In all cases, even in pots in which only one female midge had been placed, oviposition took place followed by the development of galls and, with the exception of one pot in which the plant died, adult midges subsequently emerged. These breedings may be considered as the controls.

C. Leucanthemum L. (ox-eye or dog daisy). In January 1938, 14, 11, 9, 8, 11 and 9 females were introduced into six pots containing *C. Leucanthemum* plants in the laboratory. One female was observed ovipositing on a stem just behind a leaf petiole.

[1] Parasites were bred from both samples. Those which were reared from the Worthing area were kindly identified by Dr C. Ferrière as *Eutelus diffinis* Walker.

Larvae subsequently hatched but no galls were formed. In late March and early April two more attempts to induce breeding on this plant using 3 and 5 female midges respectively were made, this time the plants being kept in the greenhouse. No oviposition was observed and no galls or adults developed. In May, 4 females were placed in a cage over an ox-eye daisy plant in the laboratory and 7 females were also put in each of two similar pots in the greenhouse. In one of the cages in the greenhouse oviposition was observed. This took place in the open blossom among the yellow disk florets, between the involucral bracts of open flower-heads and in the axils of the leaves on the flower stems. However, again no galls or adults developed. In September two more pots were set up, each with 8 female midges. No oviposition, gall formation or adults were subsequently observed.

Thus, having used 104 females on 17 different plants in January, March–April, May and September both in the laboratory and in the greenhouse, oviposition was observed on only two plants, and in no case did galls or adults develop.

C. Parthenium Bernh. In January two pots of this plant were set up in the laboratory and 21 and 12 female midges respectively were used. Oviposition readily took place in each pot in the terminal leaf buds and on the stems of the plant. Later small pustules developed on the leaves and corrugations on the stems. Young larvae were dissected out of both the pustules and the corrugations. No adults developed. In April, 3 females were placed on a plant in the greenhouse. No oviposition was observed, no gall formation resulted and no adults emerged. In May, four pots of this plant were set up in the laboratory using 4, 4, 5 and 6 female midges respectively and one pot in the laboratory using 4 females. In one pot in the greenhouse and in the pot in the laboratory, oviposition was observed in the terminal leaf buds but no galls or adults developed. In September two more plants were used in the greenhouse, 7 and 8 female midges being placed on them. No egg-laying was observed and no galls or adults resulted.

Thus, having used 74 females on 10 different plants in January, April, May and September both in the laboratory and in the greenhouse, oviposition was observed to take place on four plants, galls were formed on two plants, but adults were never obtained.

C. indicum L. Plants of this species were kindly supplied by the Director of the Royal Botanic Gardens, Kew. Two plants were subjected to trial in January in the laboratory, 1 and 7 female midges being used. In each case, egg-laying took place immediately on the young leaves and buds. Unfortunately, the plants died very quickly before galls had time to develop. In April two more plants were used in the greenhouse, 1 female midge being placed on each plant. Oviposition at once took place, galls developed on the leaves and adult midges of generation 1 duly emerged. In May, 2 females were placed on a further plant in the laboratory, egg-laying took place but again the plant died prematurely. At the same time two further plants were used in the greenhouse and 2 and 1 female midges were used respectively. Egg-laying took place in each pot, galls developed on the leaves (Pl. XXII, fig. 1) and leaf petioles and adults emerged successfully.

Thus having used 15 females on 7 different plants in January, April and May both in the laboratory and in the greenhouse, oviposition took place immediately on all seven plants, while galls formed on and adults emerged from four plants. Three plants used in the laboratory died before the galls had time to develop.

C. cinerariaefolium Vis. Plants of this species were obtained from the Ministry of Agriculture's Plant Pathology Laboratory at Harpenden. In January two plants were subjected to trial in the laboratory using 10 and 5 females respectively. Eggs were found in the uncurled leaf segments at the extremities of the leaves on one plant. No galls or adult midges developed. In May, one further plant was used in the laboratory and 4 female midges were placed on it. Egg-laying at once took place. The females appeared very keen to lay eggs in the parts of the leaves which were uncurled, while 1 female laid in an unopened flower bud. No galls or adults however developed. In May, also, two plants were tested in the greenhouse using 2 and 4 female midges. Egg-laying on one plant was observed, again in the uncurled portions of the leaves, but no galls or adults developed.

Thus, having used 25 females on 5 different plants in January and May both in the laboratory and in the greenhouse, oviposition was observed on three plants, but in no case did galls or adults develop.

C. coccineum Willd. (*roseum* Adam.). One plant of this species, obtained from a florist, was subjected to trial in the greenhouse in May using 4 female midges. Egg-laying took place in the opening flower-heads and among the involucral bracts. No galls or adults developed.

C. rubellum Sealy (*erubescens* Hort.). Plants of this species were obtained from the Royal Horticultural Society's Gardens, Wisley, through the kindness of Mr G. Fox Wilson. In May, two plants were subjected to trial in the greenhouse using 1 female midge per plant. In both cases oviposition took place at once on the extremities of the shoots, galls subsequently developed on the leaves (Pl. XXII, fig. 2), and adult midges duly emerged.

Two other plants of what apparently is this species were obtained from Miss A. Dixon, who had received the plants from Mr H. L. Jones. He in turn had received them from the Rev. D. A. Jones, Vicar of Rhuddlan, who obtained the plants from north Russia. 5 or 6 female midges were placed on each plant in the greenhouse in May. Oviposition took place immediately among the buds or shoots, galls subsequently developed and later adult midges emerged.

Thus, using 13 female midges on 4 different plants, from two localities, in the greenhouse during May, egg-laying was observed on each plant and later galls and adults successfully developed on each.

C. maximum Raymond (Shasta daisy). Plants of this species were obtained from the Royal Horticultural Society's Gardens, Wisley, again through the kindness of Mr G. Fox Wilson. One plant was tested during May in the laboratory and three in the same month in the greenhouse, using 5, 2, 4 and 3 female midges respectively.

Egg-laying was observed on the buds and young shoots on the plant in the laboratory and on one plant in the greenhouse. No galls or adult midges developed.

C. uliginosa Pers. One plant of this species was under trial in the greenhouse during May, 7 female midges being used. Oviposition took place immediately, but no galls or adults developed.

C. coronarium L. Two plants were subjected to trial during May in the greenhouse, 4 and 3 female midges being used. Oviposition took place among the young leaves just below the flower buds on both plants. No galls or adults developed.

C. frutescens Thunb. Two plants of this species, using 9 and 7 female midges respectively, were under trial during May in the greenhouse. Egg-laying was observed on each plant but again no galls or adult midges developed.

C. Zawadskii Herbich. Two plants of this species were obtained from the Royal Horticultural Society's Gardens at Wisley, through Mr G. Fox Wilson, and were tested during August in the greenhouse, using 5 and 10 female midges. No egg-laying was observed and no galls or adults developed.

C. azaleanum Hort.[1] A plant of this species was obtained from a local florist and 2 female midges were put on it in May in the greenhouse. Egg-laying took place at once on the developing shoots and subsequently typical galls and adults developed.

C. Korean Apollo. One plant obtained from a local florist was subjected to trial in May in the greenhouse. Oviposition took place immediately and typical galls and adults duly developed.

A summary of these results on different species and varieties of *Chrysanthemum* is given in Table IV. The chrysanthemum midge of commercial chrysanthemums was reared successfully on *C. indicum*, *C. rubellum*, *C. azaleanum* and *C.* Korean Apollo. Oviposition, however, was observed on all thirteen species and varieties used with the exception of *C. Zawadskii*, and it is possible that eggs may have been laid on this species also and remained unobserved. On the other hand, galls or malformations occurred only on *C. Parthenium* in addition to the species on which the midge bred successfully. In no case did galls develop on the roots, but only on the leaves and stems.

(ii) *Other plants*

Matricaria inodora (corn feverfew). Two plants were tested in the laboratory during January, 11 females being placed on each plant. Egg-laying took place in the open flower-heads on each plant, but no galls or adult midges developed. Another

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[1] A letter from the Director of the Royal Botanic Gardens contains the following remarks after stating that they could find no trace of the name *C. azaleanum*. "I find, however, that there is a plant in the Trade which is listed under the name *Chrysanthemum indicum* var. *azaleoides*, and this appears to be the same plant as the one you sent us under the name *azaleanum*. Your plant is not a true variety of *indicum* though it may have some *indicum* blood in its pedigree; it does not appear to be anything more than one of the varieties or forms of the garden Chrysanthemums, which, of course, have some *indicum* blood in them. A specific Latin epithet should, of course, not be given to a horticultural variety as it leads to much confusion and misunderstanding."

Table IV. *Summary of host range experiments on chrysanthemum midge* 1938. *I. Species and varieties of* Chrysanthemum

Plant	No. of females used	No. of plants	Egg-laying on x plants	Galls formed on x plants	Adults obtained in x pots
C. Leucanthemum L.	104	17	2	Nil	Nil
C. Parthenium Bernh.	74	10	4	2	Nil
C. indicum L.	15	7	7	4	4*
C. cinerariaefolium Vis.	25	5	3	Nil	Nil
C. coccineum Willd. (*roseum* Adam.)	4	1	1	Nil	Nil
C. rubellum Sealy (*erubescens* Hort.)	13	4	4	4	4
C. maximum Raymond	14	4	2	Nil	Nil
C. uliginosa Pers.	7	1	1	Nil	Nil
C. coronarium L.	7	2	2	Nil	Nil
C. frutescens Thunb.	16	2	2	Nil	Nil
C. Zawadskii Herbich.	15	2	Nil	Nil	Nil
C. azaleanum Hort.†	2	1	1	1	1
C. Korean Apollo	3	1	1	1	1
Autumn chrysanthemums	Hundreds	Many	100%	100%	All save one

* Three plants died before gall formation.   † See footnote on p. 333.

plant was tested during March and early April in the greenhouse using 5 female midges; oviposition was not observed and no galls or adults developed. In May, three further plants were subjected to trial; one in the laboratory and two in the greenhouse using 6, 2 and 4 female midges. Again no oviposition was observed and no galls or adults developed.

Thus using 39 females on six different plants both in the laboratory and in the greenhouse in January, March–April and in May, oviposition was observed on two plants but in no case did galls or adults develop.

This is interesting in view of Miss H. Dombrowski's record of finding *Diarthronomyia hypogaea* on *Matricaria inodora* and *M. chamomilla* (Dombrowski, 1936).

Anthemis nobilis (common chamomile). Plants of this and the next species were obtained from the Director of the St Ives Research Station, Bingley. One plant, using 7 female midges, was subjected to trial during January in the laboratory. Oviposition was observed, but no galls or adults developed. In May one other plant was used in the laboratory with 4 female midges and three plants, involving in each case 4 female midges, were used in the greenhouse. In no case was egg-laying seen, nor did galls or adults develop.

Anthemis Cotula (stinking mayweed or chamomile). One plant using 7 females was tested during January in the laboratory. Eggs were laid on the growing points. No galls or adults developed.

Achillea Millefolium (yarrow). Oviposition immediately occurred among the developing leaf buds of one plant which was given a trial using 11 females during January in the laboratory. No galls or adults developed.

Senecio vulgaris (groundsel). Oviposition took place on the stems of one plant which was given a trial using 11 females during January in the laboratory. No galls

or adults developed. This plant was in the same pot as the yarrow and the midges, having the choice of laying on either, oviposited on both.

In addition, one plant each of *Fragaria* sp. (strawberry), *Plantago lanceolata* (ribwort plantain), *Ranunculus repens* (creeping buttercup) and *Cheiranthus* sp. (wallflower) were tested all together in one pot in the laboratory, using 8 females. No signs of oviposition were observed, no galls were formed and no adults developed.

A test of the susceptibility of an *Artemisia* sp., on which a *Diarthronomyia* species of midge[1] was found breeding in Essex by Mr D. C. Thomas, was also made in July in the laboratory. Twenty-one females of the chrysanthemum midge were given the opportunity of ovipositing, but not one was observed to do so. No galls developed.

A summary of these results of experiments using plants other than *Chrysanthemum* spp. is given in Table V.

Table V. ᐧ *Summary of host range experiments on chrysanthemum midge* 1938. II. *Plants other than* Chrysanthemum *spp.*

Plant	No. of females used	No. of plants	Egg-laying on x plants	Galls formed on x plants	Adults obtained in x pots
Matricaria inodora	39	6	2	Nil	Nil
Anthemis nobilis	23	5	1	Nil	Nil
Anthemis Cotula	7	1	1	Nil	Nil
Achillea Millefolium	11	1	1	Nil	Nil
Senecio vulgaris		1	1	Nil	Nil
Fragaria sp.	8	1	Nil	Nil	Nil
Ranunculus repens		1	Nil	Nil	Nil
Plantago lanceolata		1	Nil	Nil	Nil
Cheiranthus sp.		1	Nil	Nil	Nil
Artemisia sp.	21	1	Nil	Nil	Nil

DISCUSSION

The experiments described show that the chrysanthemum midge will oviposit on a number of different species of *Chrysanthemum* and other plants belonging to the same family in addition to commercial chrysanthemums. There were, however, always commercial chrysanthemums near the experimental pots and if the presence (smell) of commercial chrysanthemums is a stimulus, this might account for the oviposition observed.

On one species only of *Chrysanthemum* did galls form followed by no adult development. It would appear, therefore, that in most cases the very young larvae could not establish themselves on the plants.

About 400 female midges were used in these experiments so that it may rightly be maintained that, if only a small fraction of a population of

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[1] The species of *Diarthronomyia*, referred to above, which was found breeding on *Artemisia* was given the opportunity of ovipositing on September White variety of commercial chrysanthemum. Thirty-three females were used in the insectary. A few eggs were laid on the developing shoots but no galls developed and no adults emerged.

chrysanthemum midges can survive on plants other than commercial varieties of chrysanthemum, these tests are not conclusive. Further, it has not been possible to make experiments with *Diarthronomyia hypogaea* F.Lw. from wild *Chrysanthemum* spp.

A knowledge of gall midges as a group, however, supports the view that it is not impossible for species to be differentiated on such biological criteria, and the experiments lend support to the view that the chrysanthemum midge of commercial chrysanthemums is at least a distinct biological species from *Diarthronomyia hypogaea* of wild *Chrysanthemum* spp.

Summary and conclusion

The chrysanthemum midge of commercial chrysanthemums was found to breed on *Chrysanthemum indicum* (one of the species from which the autumn commercial chrysanthemums have been derived), *C. rubellum*, *C. azaleanum* (=*C. indicum* var. *azaleoides*) and *C.* Korean Apollo.

Oviposition took place and galls developed on *C. Parthenium*, but no adults developed.

Oviposition took place also on *C. Leucanthemum*, *C. cinerariaefolium*, *C. coccineum*, *C. maximum*, *C. uliginosa*, *C. coronarium* and *C. frutescens*, but no galls were formed or adults developed.

Oviposition was not observed on *C. Zawadskii*; no galls or adults developed.

Oviposition also took place on *Matricaria inodora*, *Anthemis nobilis*, *A. Cotula*, *Achillea Millefolium* and *Senecio vulgaris*. No galls or adults developed on these plants.

Other plants tested included *Fragaria* sp., *Ranunculus repens*, *Plantago lanceolata*, *Cheiranthus* sp. and *Artemisia* sp. No oviposition or gall formation occurred, and no adults developed.

Another species of *Diarthronomyia* which breeds on *Artemisia* sp. laid eggs on September White variety of chrysanthemum but no galls or adults developed.

These experiments support the view that the midge of commercial chrysanthemums is distinct from *Diarthronomyia hypogaea* of wild *Chrysanthemum* spp.

IV. THE BLACK-CURRANT LEAF MIDGE

Introductory

In 1891 Rübsaamen (1891) described this midge, *Dasyneura tetensi* Rübsaamen, which he had bred from pale yellow larvae found in folded and twisted leaves on the terminal shoots of black currant (*Ribes nigrum*).

Later, the same authority (1912) stated that, having reared midges from similarly contorted leaves of gooseberry (*R. Grossularia*), he was convinced that they were the same species, namely *Dasyneura tetensi* Rübsaamen. With the solitary exception of a record by Bagnall & Harrison (1918) stating that they had found *D. tetensi* on gooseberry as well as on black currant in Durham, all other published accounts of *D. tetensi* refer to black currant as being the host plant.

On the other hand, a much less well known midge, *D. ribicola* Kieffer, has been recorded from folded leaves of gooseberry (Kieffer, 1909), but the adults of this species have not yet been described.

A similar crinkling and folding of the leaves is made by the larvae of *D. plicatrix* H.Lw. on various species of wild and cultivated *Rubus*, including blackberry (*R. fructicosus*), American blackberry, dewberry (*R. caesius*) and raspberry (*R. Idaeus*).

Life cycle on black currant

In some years in this country three generations can occur in the course of twelve months. The larvae overwinter in the soil and the first flight of midges occurs in April, May and early June. The larvae appear in the terminal leaves in late May and June. The second flight of adults takes place in late June and early July and the third in late July and August. The female midges lay their eggs on the growing tip and the lateral buds. As a consequence the leaves, instead of unfolding normally, become more and more folded and crinkled. Pupation takes place in the soil, and when the crinkled leaves turn black the larvae, having become full grown, are on the point of leaving the plant for the soil.

Host plant and cross-mating experiments

In 1930 (Barnes, 1931 *b*) tests were made to discover whether midges reared from black currant collected in Kent would oviposit on black currant, red currant, gooseberry and blackberry. Cages were set up containing black currant and wild blackberry shoots; blackberry shoots alone; black currant, red currant and gooseberry shoots; and black currant shoots alone. In each cage containing black currant the midges were seen to lay very readily on the black currant, but in no case was oviposition observed on blackberry, red currant or gooseberry.

In 1937 the writer repeated his 1930 experiments, this time on growing plants of black currant, gooseberry and wild blackberry, each in separate cages. There was thus no choice left for the midges. Midges reared from larvae obtained on black currant in Kent were used. Ovi-

position took place immediately on both plants of black currant, but no egg-laying was seen on either the gooseberry or blackberry.

Attempts were also made in 1937 to cross-mate midges (*Dasyneura tetensi*) reared from black currant with midges (*D. plicatrix*) reared from blackberry. When virgin females of *D. tetensi* were placed in tubes with males of *D. tetensi*, their ovipositors were extruded to their full extent and were waved about. The males were excited: the antennae vibrated, their wings were set in motion and the midges rushed up and down the tubes until they reached a female when mating took place.

When males of *D. plicatrix* were placed in tubes containing virgin *D. tetensi* females, the latter did not extrude their ovipositors and the males did not become excited and merely ignored the females.

When males of *D. plicatrix* and *D. tetensi* were placed in tubes containing virgin females *D. tetensi*, the last named extruded their ovipositors and the male *D. tetensi* became excited, whereas the male *D. plicatrix* remained unmoved.

In 1938 further attempts were made to induce midges, reared from larvae obtained on black currant in Kent and Hampshire, to oviposit on gooseberry. Three bushes each of gooseberry and black currant were used. Infected leaves of black currant containing larvae were placed under each of the bushes which were grown in pots and covered with muslin cages. During the period the midges were emerging observations were made frequently. Egg-laying was seen on several occasions on each of the black-currant bushes, but no oviposition was seen at all on any of the gooseberry bushes.

In addition, a few females were placed in a cage containing flowering currant (*Ribes sanguineum*) shoots. No egg-laying was observed. It is hoped to repeat this particular trial at some future date.

Discussion and conclusions

There are three very closely allied midges whose larvae cause the leaves of *Rubus* spp., *Ribes nigrum*, and *R. Grossularia* to remain folded and become twisted and crinkled. They are *Dasyneura plicatrix* H.Lw. on blackberry, *D. tetensi* Rübsaamen on black currant, and *D. ribicola* Kieffer on gooseberry. The adults of *D. ribicola* have not yet been described and the writer has had no opportunity of using material of this species.

D. plicatrix and *D. tetensi* are, morphologically, exceedingly difficult to separate but experiments made in 1937 have shown that these species will not cross-mate. *D. plicatrix* is recorded as living on *Rubus* spp. and

it has been reared on several occasions by the writer on plants of this genus. *Dasyneura tetensi* is recorded from Germany, Finland and the counties of Kent, Hampshire, Lancashire, Essex and Durham in this country. With the exception of one German and one English record of the larvae being found on gooseberry, all the records are from black currant. Confirmation of this is to be found in specimens in the writer's collection from Finland, Kent, Essex and Lancashire, in each case from black currant.

Whereas it has been possible in experiments to breed successfully *D. tetensi* on black currant on every occasion attempted, not once has the writer been able to observe oviposition on gooseberry, red currant, flowering currant or blackberry. On these latter plants in no case did the typical contortion of the leaves appear nor did adult midges develop. These experiments were carried out in three different years (1930, 1937 and 1938) and in each year fresh wild material from black currant in Kent was used. In 1938 material from Hampshire was also used.

In conclusion, therefore, it appears unlikely that *D. tetensi* Rübsaamen from black currant can breed on gooseberry, and it would seem best to regard *D. tetensi* as restricted to black currant and the two records of this species being found on gooseberry as probably referring to *D. ribicola* Kieffer, which was originally found in folded leaves of gooseberry. The question, however, cannot be finally settled until specimens of *D. ribicola* on gooseberry have been found, experiments made to induce them to oviposit and breed on black currant and attempts made to cross-mate midges from gooseberry with others from black currant.

V. THE HAWTHORN STEM MIDGE

Introductory

In August 1937 Mr H. C. F. Newton noticed very common and widespread damage to hawthorn hedges in Shropshire, Staffordshire and Warwickshire. Young branches were being killed and became conspicuous owing to the withered leaves turning brown. Gall-midge larvae were present in the stems and death of the branches occurred above the points attacked. The bark at the infested areas flaked off easily, revealing clusters of pink larvae. The colour of the larvae was typical of the genus *Thomasiniana* as represented by *T. oculiperda* Rübsaamen, the red bud borer, and *T. theobaldi* Barnes, the raspberry cane midge. Material was received on 13 August and one male and one female emerged on 8 and

22 September respectively. These provided confirmation of the tentative identification that the damage was caused by a species of *Thomasiniana*.

Dr F. W. Edwards informs the writer that some years ago he noticed similar dying of shoots with midge larvae present on hawthorn hedges in Hertfordshire. A single larva of this species was found at Kinsbourne Green, Harpenden in September 1937 (Cecid. 3185).

The only references found in the literature to midges infesting hawthorn stems include one by Rudow (1875), who stated that attacked branches were swollen, the epidermis becoming brown and flaking off easily. Bergenstamm & Loew (1877) referred to Rudow's note, and Schlechtendal (1891) mentioned it again. No name was suggested for the insect causing this malformation, but it appears possible that these authors were dealing with the midge under discussion.

Life history

There appear to be two generations during the year. As has already been stated two adult midges emerged at Harpenden in September 1937 from material collected in Staffordshire on 12 August. Emergence started again on 26 May 1938 and continued until 13 July, the peak of emergence being about 11 June.

Midges were ovipositing in experiments from 3 to 15 June and the second generation of adults appeared on the wing from 1 to 14 August. The time of development for the summer generation was 56–62 days.

Pupation takes place in the soil. Emergence of the midges was noticed to occur regularly between 4.30 and 8.30 p.m. (British summer time). Mating lasted just over 2 min. and the fertilized females lived about 5 days, though in one case a female was observed still ovipositing on the eighth day after emergence. Normally oviposition by a single female is spread over several days.

Egg-laying was only observed to take place in artificial and natural slits in the bark of hawthorn stems; where the stems were undamaged no oviposition occurred. Egg-laying started about 7 p.m. and continued at least until dark (in early June). During the earlier part of the day the midges remained settled on the stems of hawthorn and the sides of the cages.

In two breeding experiments in which 3 females and 1 female were used, the number and sexes of the progeny were 3 males and 35 females and 19 females only respectively; an indication that unisexual progeny occur in this species. The sex ratio of bulk rearing was 46 : 54, as would be expected.

Only two individual parasites were reared, while 252 midges emerged from wild material.

Similarity between the red bud borer, raspberry cane midge and the hawthorn stem midge

Morphologically, the red bud borer (*T. oculiperda* Rübsaamen), the raspberry cane midge (*T. theobaldi* Barnes) and the hawthorn stem midge are very similar. On one occasion a male *T. oculiperda* mated with a female *T. theobaldi*, but this has never been repeated. The colour of the larvae is also a peculiar pink in each species.

Biologically, the three midges, so far as is known, are very similar. The adults all rest in a characteristically flattened attitude. The females of *T. oculiperda* oviposit under grafts and, where no grafts are available, in slits made artificially in the stems of rose trees; those of *T. theobaldi* lay under the broken skin of raspberry canes; while those of the hawthorn stem midge lay their eggs in slits artificially made in the branches of hawthorn as well as in any naturally occurring breaks in the bark.

Emergence of the adults of *T. oculiperda* took place regularly in the evenings from about 4.30 to 8.30 p.m. (British summer time), as did also those of the hawthorn stem midge. Egg-laying was observed in both species to take place in the evenings, at any rate up to dark. No information on these points is available concerning *T. theobaldi*.

In mass breedings the sex ratio of all three midges is roughly 50 : 50, but slightly in favour of the females. *T. oculiperda* was shown (Barnes, 1931 *b*) to reproduce sexually by means of unisexual families, and there is now evidence which indicates that the hawthorn stem midge does the same. Nothing is known in this respect concerning *T. theobaldi*.

T. oculiperda has three generations a year. In 1930 the first flight was on the wing during the latter part of May and nearly all June, the second in July and early August, and the third in late August and September. *T. theobaldi* so far as is known has one generation a year: the adults being on the wing—in 1929 and 1930—in the latter part of May, throughout June and the early part of July. The hawthorn stem midge has two generations a year. The first flight in 1938 appearing from late May, throughout June and in early July, while the second was on the wing during the first fortnight of August. A few emergences also took place in September in 1937.

All the above information has been derived from experiments carried out by the writer at the Rothamsted Experimental Station.

Host plant experiments

In view of the similarity between the biology of these three midges and the difficulty in separating the adults on morphological grounds, it was decided to carry out experiments concerning the host plant range.

Previously (Barnes, 1931 *b*) unsuccessful attempts were made to induce *T. oculiperda* to oviposit and live on "Lloyd George" variety of raspberry, which is one of the three varieties known to be attacked by *T. theobaldi*: the others are Bath's Perfection and Reader's Perfection.

In 1938, trials were made with the hawthorn stem midge using three varieties of rose—Southport, Lady Pirie and Inchiquin—three pots of "Lloyd George" variety of raspberry, and four pots of common hawthorn. In every case slits in the stems were made with a scalpel.

In two pots of hawthorn only one female midge was used in each, in the third 3 females and in the fourth 10 females. Egg-laying was seen in each pot of hawthorn several times. The female midges were observed walking up and down the stems till they found a slit and then oviposition took place in the slits. Later examination of the slits revealed larvae in each pot. Adults emerged successfully in two pots, in the third a layer of sand was placed over the soil and no emergence took place, and in the fourth there was very serious overcrowding and the bush died while the larvae were still small.

In both the pots containing Southport rose trees 4 female midges were used, in the Lady Pirie pot 1 female was placed and in the Inchiquin pot 3 females. Although the pots were under continual observation until dark, no oviposition was seen, though one female, which was empty of eggs, was taken from one of the Southport pots after it had been in the cage for 48 hr. No larvae were found in any of the slits and no adult midges subsequently developed.

In one of the raspberry pots 1 female was used and, in the other two, 3 female midges. Here again no oviposition was observed and the females all appeared full of eggs until they died. No larvae were found in the slits in the stems and no adult midges subsequently developed.

Conclusions

The three midges, *T. oculiperda* Rübsaamen, *T. theobaldi* Barnes, and the hawthorn stem midge, form a group of midges which are morphologically very difficult, if not impossible, to separate when one is dealing with numerous specimens. Biologically they have many common attributes but there are differences, e.g. the number of generations and

host plants. Attempts have been made without success to extend the host plant range of two of the three species under consideration.

The evidence, therefore, indicates that the three midges should be regarded as distinct species and this necessitates describing the hawthorn stem midge as a new species.

The name *Thomasiniana crataegi* sp.n. is proposed for this midge and a description follows.

Description of Thomasiniana crataegi *sp.n.*

Male. Length $1\frac{1}{2}$–$2\frac{1}{2}$ mm. Antennae: $2+12$, first and second flagellar segments fused, each flagellar segment consisting of basal subglobular node bearing one ring of circumfila with regular loops reaching nearly to the base of distal node and a whorl of stout long setae twice as long as loops, and a distal elongated node bearing two rings of circumfila, in the basal ring the loops extend as far as the points of attachment of the distal ring of circumfila, those of distal ring longer, reaching the basal node of the next segment, in addition a whorl of long stout setae distally, slightly longer than the loops of the distal ring of circumfila, these loops being the longest of the three rings; the basal and distal nodes separated by a stem and each distal node bears a distinct neck; the stem of the 3rd flagellar segment is about three-fifths as broad as long and the neck is about twice as long as broad, the neck slightly longer than the stem; stem of 10th flagellar segment about 3 times as long as broad, neck about 4–5 times as long as broad; distal node of 12th flagellar segment bears a distinct and nearly globular elongation. Palpi: proximal segment slightly longer than wide, second about $2\frac{1}{2}$–3 times as long as wide, third usually about the same, sometimes slightly longer and narrower, distal segment usually distinctly longer and slightly narrower, about 5 times as long as wide. Wings: rather dark owing to being covered with many fine hairs, 3rd vein reaching margin of wing at the tip. 5th vein forked, the two branches forming almost a right angle, the lower branch short. Legs pale with dark hairs, claws bent at right angles, with basal tooth, empodium small. Genitalia: basal clasp segment stout, twice as long as basal width, narrowing distally; distal clasp segment tapering; dorsal lamella with deep narrow V-shaped emargination, each lobe broadly rounded; lower lamella slightly longer than dorsal lamella, with rather shallow U-shaped emargination, each lobe narrowly rounded.

Cotypes: Cecid. 3715–19, 3723–5 and 3748–54.

Other specimen: Cecid. 3223.

Female. Length 2–3 mm. Antennae: $2+12$, first and second flagellar segments fused, each flagellar segment cylindrical with short transverse neck, with two almost regular whorls of long stout setae, the setae in the basal whorl much longer than those in the distal, two rings of applied circumfila united by a longitudinal thread, length of 3rd flagellar segment about $2\frac{1}{2}$ times as long as wide, length of 12th flagellar segment about 3 times as long as wide including the distal prolongation which bears long setae. Palps about as in male. Abdomen salmon pink-red, ovipositor long, very extensile, lamelliform, the paired lamellae elongate ovoid, with long fine setae, the basal lamella very small. Otherwise about as in male.

Cotypes: Cecid. 3732–43 and 3745–7.

Other specimen: Cecid. 3224.

Larvae. Salmon pink in colour, breast bone bifid, rounded lobes, shallow rounded emargination, 3–4 mm. long when full grown.

Cecid. 3163–4 and 3185.

Habitat. Under bark of hawthorn twigs.

Locality. Staffordshire, Shropshire, Warwickshire and Hertfordshire.

VI. SUMMARY AND CONCLUSIONS

Emphasis is laid on the need for using biological in addition to morphological characters, in the separation of closely allied species of gall midges.

An analysis is given of the recorded host plant range of species of the genus *Asphondylia*, showing that the great majority of species, 103 out of 133, are recorded from only one species of plant.

Four studies concerning gall midges of economic importance are presented in support of the plea for more intensive biological studies.

The first deals with three supposed species which make terminal leaf galls on various species of *Arabis*. As a result of the study it is concluded that only one true species causes this damage. The species is *Dasyneura alpestris* (Kieffer) de Meijere, while *D. schneideri* Rübsaamen and *D. arabis* Barnes are synonyms. The midge involved attacks certain species of the subgenus *Euarabis* and one other species which may belong to the subgenus *Lomaspora*.

The second study deals with the chrysanthemum gall midge, *Diarthronomyia* sp. Its distribution on commercial chrysanthemums is traced from its initial record as a pest in the United States of America and Canada in 1915. It appeared in England in 1927 and again in 1936. on both occasions the infestation being traceable to chrysanthemum varieties imported from the U.S.A. More recently it has appeared in Denmark (1934), in Sweden (1935), in Finland (1936) and in Northern Ireland (1937). Experiments on the host plant range are described. Oviposition, only, took place on nearly all the *Chrysanthemum* spp. subjected to trial. Galls were formed on *C. Parthenium*, but no adults developed. The midge was bred successfully only on commercial varieties of autumn chrysanthemums, *C. indicum* L., *C. rubellum* Sealy, *C. indicum* var. *azaleoides* (*C. azaleanum* Hort.) and *C.* Korean Apollo. In addition, eggs were laid on such plants as *Matricaria inodora, Anthemis nobilis, A. Cotula, Achillea Millefolium* and *Senecio vulgaris*: in these cases no galls were formed. The conclusion is reached that the midge of commercial chrysanthemums should be regarded as distinct and separate from *Diarthronomyia hypogaea* F.Lw. of wild *Chrysanthemum* spp. recorded from Central Europe.

The third study involves three species of the genus *Dasyneura*, whose larvae cause the leaves of *Rubus* spp., *Ribes nigrum* and *R. Grossularia* to become twisted and crinkled. Attempts to induce *Dasyneura tetensi* Rübsaamen of black currant to attack gooseberry always failed. *D. tetensi* would not mate with *D. plicatrix* H.Lw. of blackberry. In spite of two records in the literature of *D. tetensi* attacking gooseberry, the conclusion is reached that the three species are genuine species—*D. tetensi* Rübsaamen confining its attention to black currant, *D. ribicola* Kieffer limiting itself to gooseberry, and *D. plicatrix* H.Lw. only attacking species of *Rubus*.

The fourth study describes the biology of the newly discovered hawthorn stem midge and compares its bionomics with those of the red bud borer (*Thomasiniana oculiperda* Rübsaamen) and the raspberry cane midge (*T. theobaldi* Barnes). It is concluded that the three midges are distinct species and a description is added of the hawthorn stem midge, to which the name *Thomasiniana crataegi* sp.n. is given.

In addition to those persons whose assistance has already been acknowledged in the course of this paper, the writer wishes to take this opportunity of expressing his gratitude to Miss F. L. Stephens, British Museum (Nat. Hist.), who examined critically the manuscript and made some valuable suggestions which have been adopted. Finally, various discussions with colleagues on the Staff of the Entomology Department at Rothamsted have been of much value.

REFERENCES

References on purely botanical matters are not included in this list, but may be found in the text. References which are asterisked have not been seen in the original.

AHLBERG, O. (1938). Nya iakttagelser rörande Krysantemumgallmyggan. *Växtskyddnotiser Växtskyddsanst.*, *Stockh.*, **5**, 65–6.

ANON. (1937). The chrysanthemum midge. *J. Minist. Agric.* **43**, 1158–61.

BAGNALL, R. S. & HARRISON, J. W. H. (1918). A preliminary catalogue of British Cecidomyidae (Diptera) with special reference to the gall-midges of the north of England. *Trans. ent. Soc. Lond.* **65**. 346–426.

—— —— (1921). New British Cecidomyidae. I. *Ent. Rec.* **33**, 151–5.

BALDRATI, J. (1900). Appunti di Cecidiologia. *Nuovo G. bot. ital.* **7** (n.s.), 5–95.

BARNES, H. F. (1931 *a*). The sex ratio at the time of emergence and the occurrence of unisexual families in the gall midges (Cecidomyidae, Diptera). *J. Genet.* **14**, 225–34.

—— (1931 *b*). Observations on gall midges affecting fruit trees. *J. S.-E. agric. Coll. Wye*, **28**, 170–7.

—— (1932). A study of the segmentation of the antennae in gall midges (Cecidomyidae). *Proc. zool. Soc. Lond.* pp. 323–34.

BARNES, H. F. (1935 a). On the gall midges injurious to the cultivation of willows. II. The so-called "Shot Hole" gall midges (*Rhabdophaga* spp.). *Ann. appl. Biol.* **22**, 86–105.

—— (1935 b). Studies of fluctuations in insect populations. IV. The *Arabis* midge, *Dasyneura arabis* (Cecidomyidae). *J. Anim. Ecol.* **4**, 119–26.

—— (1935 c). Studies of fluctuations in insect populations. V. The leaf-curling pear midge, *Dasyneura pyri* (Cecidomyidae). *J. Anim. Ecol.* **4**, 244–53.

BARNES, H. F. & THEOBALD, F. V. (1927). A new gall midge attacking *Arabis albida*. *J. S.-E. agric. Coll. Wye*, **24**, 54–5.

BERGENSTAMM, J. E. & LOEW, P. (1877). Synopsis Cecidomyidarum. *Verh. zool.-bot. Ges. Wien*, **26**, Abhandlungen, 1–104.

BOVIEN, P. (1935). Plantesygdomme i Danmark 1934. *Tidsskr. Planteavl.* **40**, 713–72.

—— (1937). Plantesygdomme i Danmark 1937. *Tidsskr. Planteavl.* **42**, 228–78.

*COTTE, J. (1912). *Recherches sur les Galles de Provence*. Thèse pharmacie Paris, Tours, lii + 240 pp., figs. 1–15. Quoted in Houard, C., *Les Zoocécidies des Plantes d'Europe et du Bassin de la Méditerranée*, **3**, 1913, 1483, fig. 1533.

*DANILTCHENKO, J. M. (1916). [Chrysanthemums and their cultivation.] Supplement to [*Progressive Horticulture and Market Gardening*, Petrograd], 31 pp. (*R.A.E.*, A, **5**, 1916, 164.)

DESHUSSES, J. & L. (1936). Diptères nuisibles aux cultures, nouveaux pour la faune suisses ou peu connus. *Mitt. schweiz. ent. Ges.* **16**, 740–9.

DOMBROWSKI, H. (1936). [On the Cecidomyid fauna of the Kamennaja Steppe, U.S.S.R.] *Trav. Inst. Zool. Acad. Sci. U.R.S.S.* pp. 409–28.

ESSIG, E. O. (1916). The chrysanthemum gall-fly, *Diarthronomyia hypogaea* (F. Löw). *J. econ. Ent.* **9**, 461–8.

FELT, E. P. (1915). A new pest, the chrysanthemum midge (*Rhopalomyia hypogaea* H.Lw.). *J. econ. Ent.* **8**, 267.

—— (1916). Chrysanthemum midge in 31st Report of the State Entomologist on injurious and other insects of the State of New York. *Bull. N.Y. St. Mus.* no. 186, pp. 51–5.

GIBSON, A. (1916). Reports on insects of the year. Division No. 1, Ottawa District. *Rep. ent. Soc. Ont.* **46**, 11–14.

—— (1917). Three important greenhouse pests recently introduced into Canada. *Rep. ent. Soc. Ont.* **47**, 111–22.

GIBSON, A. & ROSS, W. A. (1922). Insects affecting greenhouse plants. *Bull. Dep. Agric. Can.* **7** (n.s.), 1–63.

HAMILTON, C. C. (1924). The biology and control of the chrysanthemum midge (*Diarthronomyia hypogaea*, F. Loew). *Bull. Md. agric. Exp. Sta.* no. 269, pp. 1–51.

HOUARD, C. (1913). *Les Zoocécidies des Plantes d'Europe, etc.*, Paris, **3**, 1483.

KIEFFER, J. J. (1909). Contributions à la connaissance des Insectes gallicoles. *Bull. Soc. Hist. nat. Metz*, **26**, 1–35.

*LEMÉE, E. (1902). Les ennemis des Plantes, etc. *Bull. Soc. Hort. Orne*, 52 pp. (p. 35, no. 103).

LOEW, F. (1885). Beiträge zur Naturgeschichte der gallenerzeugenden Cecidomyiden. *Verh. zool.-bot. Ges. Wien*, **35**, 483–510.

—— (1888). Mittheilungen über neue und bekannte Cecidomyiden. *Verh. zool.-bot. Ges. Wien*, **38**, 231–46.

M. OF A. (1928). Insect pests of crops, 1925–7. *Misc. Publ. Min. Agric. Fish., Lond.*, no. 62, pp. 1–47.

—— (1933). Insect pests of crops, 1928–31. *Bull. Minist. Agric., Lond.*, **66**, 1–50.

M. OF A. (1936). Insect pests of crops, 1932–4. *Bull. Minist. Agric., Lond.*, **99**, 1–50.

MEIJERE, J. C. H. DE (1928). Vierde Supplement op de Nieuwe Naamlijst van Nederlandsche Diptera. *Tijdschr. Ent.* **71**, 11–83.

MILES, H. W. (1929). The chrysanthemum midge, *Diarthronomyia hypogaea* F.Lw. *Northw. Nat.* **4**, 173–5.

MOREILLON, M. (1916). Seconde contribution au catalogue des zoocécidies de la Suisse. *Bull. Soc. vaud. Sci. nat.* **51**, 143–71.

PERRIRAZ, J. (1909). Contribution à l'étude des Monstruosités chez *Thymus Serpyllium* et *Arabis alpina*. *Bull. Soc. vaud. Sci. nat.* **45**, 409–15.

RÜBSAAMEN, E. H. (1891). Neue Gallmücken und Gallen. *Berl. ent. Z.* **36**, 393–406.

—— (1896). Ueber russische Zoocecidien und deren Erzeuger. *Bull. Soc. Nat. Moscou*, **9**, 1895, 396–488.

—— (1912). Ueber deutsche Gallmücken und Gallen. *Z. wiss. InsektBiol.* **8**, 354–7.

—— (1917). Cecidomyidenstudien. VI. *S.B. Ges. naturf. Fr. Berl.* pp. 36–99.

RUDOW, F. (1875). Die Pflanzengallen Norddeutschlands und ihre Erzeuger. *Arch. Ver. Naturg. Mecklenb.* **29**, 1–96.

SCHLECHTENDAL, D. H. R. VON (1891). Die Gallbildungen (Zoocecidien) der deutschen Gefässpflanzen. *Jber. Ver. Naturh. Zwickau*, 1890, pp. 1–122.

SPEYER, E. R. (1927 a). The chrysanthemum midge. *Gdnrs' Chron.* **82**, 388.

—— (1927 b). The chrysanthemum midge. Its occurrence and control in England. *Fruitgrower*, **64**, 951–2.

—— (1928). Entomological Report. *Rep. exp. Res. Sta. Cheshunt*, 1927, 60–80.

STATENS VÄXTSKYDDSANSTALT (1936). Krysantemumgallmyggan. *Flygbl. Skogsforsoksanst., Stockh.*, **28**, 7 pp.

SUIRE, J. (1935). Sur quelques parasites des chrysanthèmes. *Bull. Soc. Hist. nat. Herault*, reprint 11 pp. (*R.A.E.* A, **24**, 1936, 289).

THOMAS, F. (1886). Suldener Phytoptocecidien. *Verh. zool.-bot. Ges. Wien*, **36**, 295–306.

TREHERNE, R. C. (1916). Insects affecting agriculturists in British Columbia during the past year. *Agric. J. Vict. B.C.* **1**, 168.

VOGLER, P. (1906). Zoocecidien von St Gallen und Umgebung. I. *Jb. naturw. Ges. St Gallen*, 1905, pp. 311–42.

WEIGEL, C. A. & SANFORD, H. L. (1920). Chrysanthemum midge. *Bull. U.S. Dep. Agric.* no. 833, pp. 1–25.

EXPLANATION OF PLATE XXII

Fig. 1. Galls of chrysanthemum midge on *Chrysanthemum indicum* L.
Fig. 2. Galls of chrysanthemum midge on *Chrysanthemum rubellum* Sealy (*erubescens* Hort.).

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AN APPARATUS FOR TESTING AND COMPARING THE BIOLOGICAL ACTION OF INSECTICIDES ON FLYING INSECTS AND A METHOD FOR SAMPLING THE CONCENTRATION OF THE ATOMIZED INSECTICIDE

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(With 8 Text-figures)

CONTENTS

INTRODUCTION

A CONSIDERABLE number of appliances and methods have been described for testing fly sprays. Such an apparatus or method should reproduce as nearly as possible the conditions likely to occur in practice, and of the published methods only those of Peet-Grady (1928) and its modifications and Richardson (1931) appear to fulfil this condition.

The Peet-Grady method has been adopted as a standard in America (see *Soap Blue Book*, 1938, pp. 145, 147, 153), but it seemed to us that Richardson's apparatus was better for a number of reasons. It is not so unwieldy, it can be cleaned more easily and a fan is used to maintain an even temperature and to distribute the insecticide. An apparatus was therefore constructed with Richardson's as a model, but with some slight alterations.

This preliminary apparatus was used to test the emulsified fly sprays, and some of the results are given to show the type of toxicity curves that were obtained. When analysed these results indicated that considerable care must be used in their interpretation.

The major fact which emerged from this work was the necessity of evolving some method of sampling the concentration of the insecticide in the free space of the chamber. If this is not done it is impossible to compare insecticides in different carriers, or to form any estimate of the effect of alteration of the degree of atomization which invariably occurs.

Some further changes were made in the apparatus to ensure that the insecticide was evenly distributed and applied, to facilitate cleaning and to eliminate any chance of toxic residues being left in the chamber. A sampling method was then worked out and tested with three insecticide carriers under a number of different conditions.

PRELIMINARY EXPERIMENTS

The following is a brief description of the first apparatus. It consisted of a rectangular box completely lined with lead and glass to facilitate cleaning. A large slow-moving fan was placed at one end to distribute the insecticide and maintain an even temperature. The apparatus was heated by woven wire resistance mats beneath the glass floor. The inner cage which contained the flies under test was of open mesh tinned gauze and measured $75 \times 45 \times 45$ cm.

The atomizer was an Aerograph "E" model air brush placed in the centre of the end opposite the fan. To avoid excessive coating of one end of the cage, this was placed against the end of the apparatus carrying the atomizer, the tip of which was inserted into the cage so that the insecticide was atomized directly into the cage and then circulated throughout the chamber.

This apparatus was used to test the effect of varying the proportion of oil-in-water emulsions of oil and 4% β-butoxy-β'-thiocyanodiethylether. The oil used was White May kerosene of the following specification: sp. gr. 0·785, flash-point (closed) 125° F., initial boiling-point 170° C., final boiling-point 275° C., percentage distilling at 200° C. = 20. The emulsifier was supplied by Universal Emulsifiers, Ltd., and consisted of inert alumina.

The test insects were *Phormia terraenovae* R.-D. which were bred in the laboratory. They were not standardized with reference to sex but were roughly of the same age.

Table 1 and Fig. 1 show the results of these experiments. Four experiments were done at each concentration and approximately 100 flies were used for each experiment.

It will be seen from the figures that, under the conditions of the experiment, increasing the percentage of oil in the emulsion caused a

Table I. *The rate of paralysis of flies* Phormia terraenovae *R.-D. caused by atomized emulsions of 4 % β-butoxy-β′-thiocyanodiethylether in water containing varying proportions of White May kerosene. The insecticide was emulsified with inert alumina and 1·5 c.c. were atomized into the test chamber.*

Interval after spraying (sec.)	Log of interval	100% oil		60% oil		50% oil		40% oil	
		% para-lysed	Probit	% para-lysed	Probit	% para-lysed	Probit	% para-lysed	Probit
60	1·7782	5	3·3551	4	3·2493	2	2·9463	2	2·9463
120	2·0792	39	4·7207	27	4·3872	15	3·9636	12	3·8250
180	2·2553	75	5·6745	62	5·3055	45	4·8743	36	4·6415
240	2·3802	94	6·5548	82	5·9154	79	5·8064	57	5·1764
300	2·4771	97	6·8808	94	6·5548	88	6·1750	67	5·4399
360	2·5563	99	7·3263	96·5	6·8119	89·5	6·2536	74	5·6433
420	2·6232	—	—	—	—	90·5	6·3106	81	5·8779
480	2·6812	—	—	—	—	—	—	85	6·0364
540	2·7324	—	—	—	—	—	—	—	—
600	2·7782	—	—	—	—	—	—	—	—
660	2·8195	—	—	—	—	—	—	—	—
720	2·8573	—	—	—	—	—	—	—	—

Interval after spraying (sec.)	Log of interval	30% oil		20% oil		10% oil	
		% para-lysed	Probit	% para-lysed	Probit	% para-lysed	Probit
60	1·7782	0	—	0	—	0	—
120	2·0792	6	3·4452	4	3·2493	2	2·9463
180	2·2553	23	4·2612	21	4·1936	12	3·8250
240	2·3802	42	4·7981	36·5	4·6549	24	4·2937
300	2·4771	59	5·2275	48	4·9498	39	4·7207
360	2·5563	64	5·3585	59	5·2275	53	5·0753
420	2·6232	70	5·5244	63	5·3319	59·5	5·2404
480	2·6812	72·5	5·5978	71	5·5534	65	5·3853
540	2·7324	75·5	5·6903	76	5·7063	69	5·4959
600	2·7782	77·5	5·7554	80·5	5·8596	72	5·5828
660	2·8195	—	—	83	5·9542	79	5·8064
720	2·8573	—	—	—	—	75	5·6745

marked increase in toxicity over a range of 10–50 %, but the increase in toxicity was much more gradual over the range 50–100 % oil.

The results were originally plotted as the percentage paralysed against the time of exposure, when sigmoid curves were obtained. However, when the percentage paralysed was converted to probits and plotted against the logarithm of the time of exposure, the points for each concentration of oil did not always lie along a straight line. They seemed, in the lower concentrations, to be best fitted by two straight lines, the break appearing towards the end of the exposure period. The lines shown are only the freehand provisional regression lines.

In these experiments, the concentration of the insecticide in the chamber is continuously falling, so that a straight line relationship

between the exposure and the number paralysed is not to be expected. The explanation of the fact that the points do not lie on a pronounced curve may be that, after the initial rapid fall, the concentration of insecticide falls off gradually so that, for a period, the ordinary straight line dosage-mortality relationship holds, but at some point in the exposure
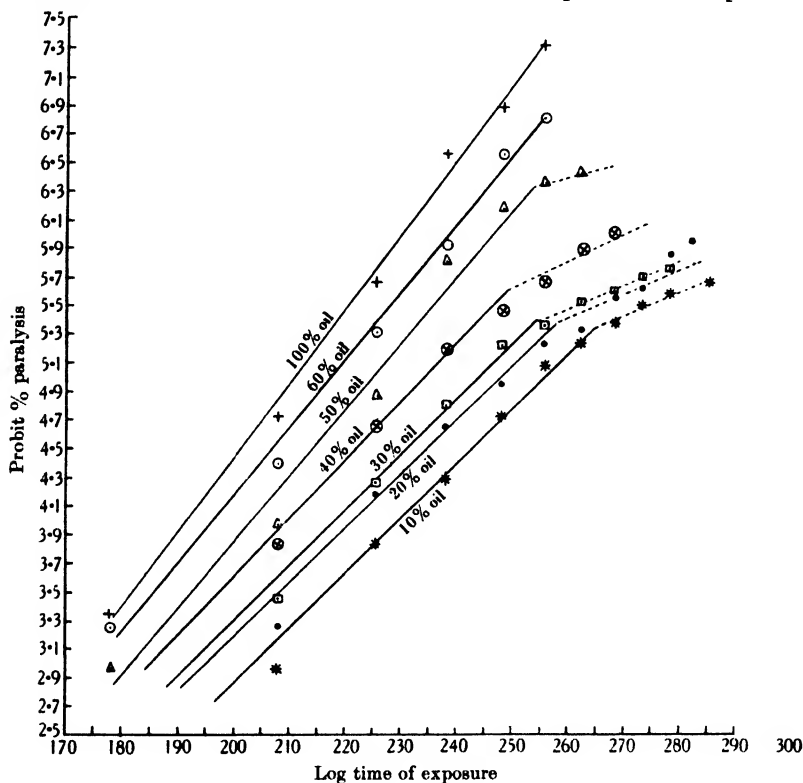
Fig. 1. Graph from Table I showing the effect of emulsions of 4% β-butoxy-β'-thiocyanodiethylether in water containing varying proportions of White May kerosene on *Phormia terraenovae* R.-D. 1·5 c.c. of insecticide atomized into the chamber.

the concentration falls below a threshold value and a break in the line occurs. The reason for no break occurring in the high percentages of oil may be, that they were atomized more finely and the concentration did not fall below the threshold value during the time of exposure allowed.

It became evident during the course of these experiments that insufficient data were available to give a satisfactory interpretation of the results. The lowering of toxicity, with decreasing percentages of oil

in the emulsion, might equally well be due to a more rapid fall in concentration of the insecticide in the chamber (due to less fine atomization) as to an actual decrease in the toxicity of the emulsion.

Further, it appeared that a method of measuring the concentration of the atomized insecticide in the chamber was essential, in order to compare insecticides in carriers of different physical properties and in order to standardize the degree of atomization with any given carrier.
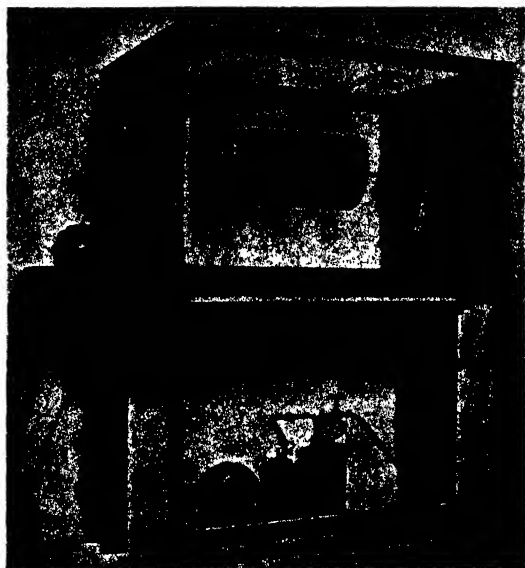
Fig. 2. Photograph of testing apparatus finally adopted.

In addition, some modifications of the apparatus were desirable. The disadvantages of the original apparatus were, first, that the flies had to be introduced before spraying and were liable to get a heavy dose of insecticide before it was properly distributed; secondly, that different parts of the cage containing the flies received very different deposits of spray so that the flies crawling on it were variously affected.

An apparatus was therefore constructed to overcome these two defects and a sampling technique was also worked out.

DESCRIPTION OF THE FINAL APPARATUS (Figs. 2, 3)

The testing chamber measured $4 \times 2\frac{1}{2} \times 2\frac{1}{2}$ ft. with a framework of $2 \times 1\frac{1}{2}$ in. deal. The back, top and hinged front were made of 32 oz. plate glass (PG) and the ends of $\frac{1}{2}$ in. 9-ply birch (W). The floor (Fg) was
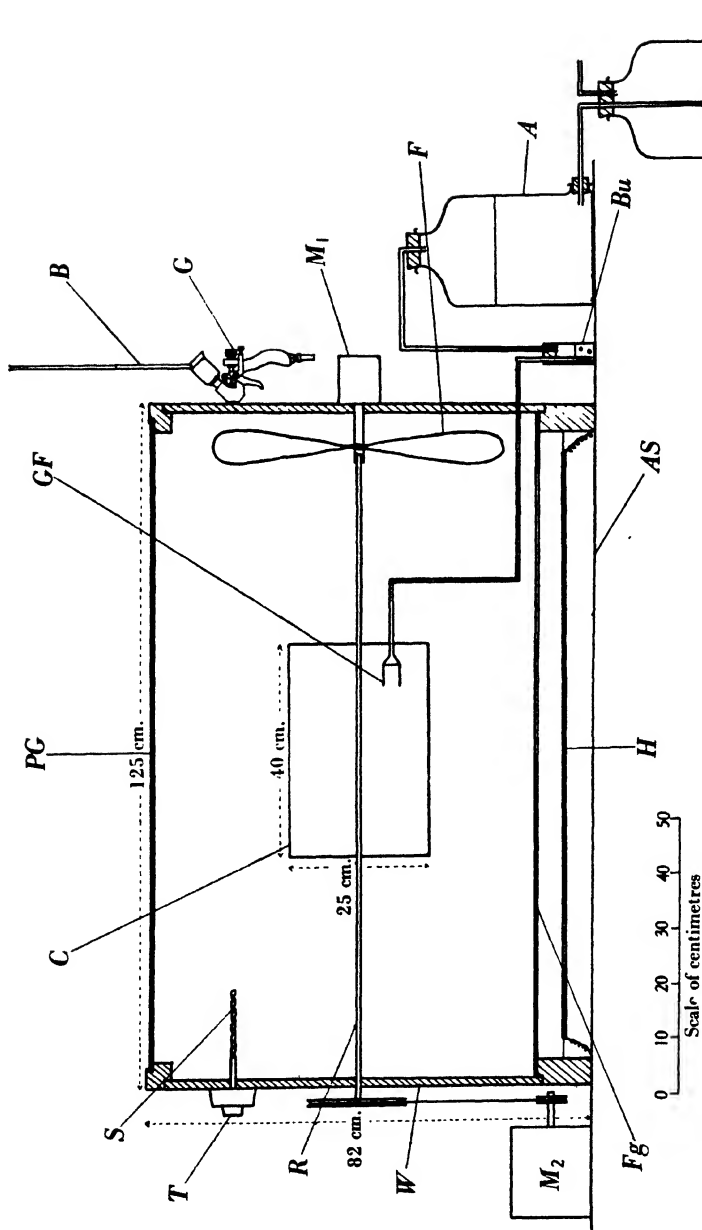
Fig. 3. Transverse section of chamber showing sampling apparatus in position.

A, aspirator; *AS*, asbestos stand; *B*, burette to measure the insecticide; *Bu*, bubbler; *C*, wire gauze insect cage; *F*, slow-moving fan; *Fg*, glass floor; *GF*, sintered glass filter; *H*, "Cressall" heating mats; *M₁*, Aerograph MP gun; *M₂*, motor to rotate insect cage; *PG*, plate glass; *R*, axle to support insect cage; *M₁*, 1/50 H.P. fan motor; *S*, bimetallic spiral of thermostat; *T*, Sunvic thermostat; *W*, ⅛ in. 9-ply birch ends.

of 32 oz. plate glass supported by ½ in. birch plywood, in which five parallel rectangular openings 3 × 4 in. were cut to expose five woven wire "Cressall" heating mats (H), each of 42 ohms resistance and wired to take 1 kW. The chamber rested on a sheet of 3/16 in. asbestos (AS). The temperature was controlled (within $\pm 1°$ C.) by a type TS Sunvic thermostat (T) fixed with a relay on the outside of the left wall with the bimetallic spiral (S) projecting into the chamber. All internal wood surfaces were covered with heavy (7 oz./sq. ft.) lead foil.

A 2 ft. diameter fan (F) was fixed centrally on the right-hand wall and was revolved in a counter clockwise direction at approximately 100 rev./min. by a 1/50 H.P. electric motor (M_1) which was outside the chamber.

The insecticide was sprayed in at a point 5 in. from the top and front on the right-hand end by a type MP Aerograph spray gun (G) with a no. 1 nozzle worked by a small air compressor shown in Fig. 2.

A cylindrical open mesh (8 to the inch) brass gauze cage (C), 10 in. in diameter and 15 in. long, was suspended in the centre of the chamber on a 3/8 in. axle (R) which pivoted in the centre of the fan and passed through the left-hand end. The cage could be slowly rotated in the reverse direction to the fan by a fractional H.P. electric motor (M_2) outside the chamber. A cage of smaller mesh is necessary if mosquitoes are used.

SPRAYING TECHNIQUE

With the chamber closed and the fan and gauze cage (C) rotating in opposite directions, a known quantity of insecticide (usually 3 c.c.) was sprayed in from burette B under a known air pressure (usually 30 lb./sq. in. recorded on the gauge of the air compressor) in a known time. The time was checked by a stop-watch. After 4 min., when the mist was distributed and the initial rapid fall in concentration had taken place, the rotation of the cage was stopped and a glass tube 2 ft. long and 1 in. in diameter, containing fifty flies, was put horizontally through a 1 in. hole in the left end of the chamber and through a hole in the left end of the cage. The flies were gently pushed out of the tube and into the cage with a perforated piston. The tube and piston were then withdrawn and the hole closed with a bung (see below for method of introducing mosquitoes).   •

The number of flies knocked down and paralysed at ½ min. intervals was counted.

When the flies had been in the mist for 15 min. the chamber was

opened, the cage removed, one end taken off and the flies gently shaken into a jar where they were left for 24 hr. with sugar solution as food and drink. The numbers completely dead and moribund at the end of that time were recorded.

The whole of the chamber and the cage was thoroughly wiped with acetone, and after airing for about half an hour the apparatus was ready for further use.

Control experiments indicated that this was a satisfactory method of cleaning the apparatus, but it would be preferable to have at least two cages, so that one could be thoroughly cleaned while the other was in use.
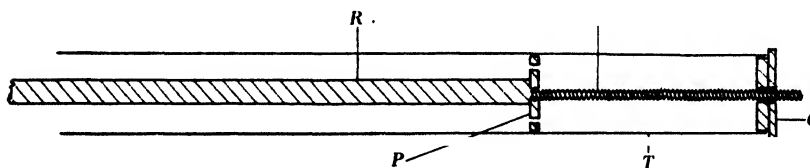
Fig. 4. Apparatus for introducing mosquitoes into the testing chamber. C, screw-on cap; P, perforated piston; R, push rod; S, mosquito chamber; T, glass tube.

Mosquitoes were found to be too fragile to be pushed along the glass tube so the arrangement shown in Fig. 4 was used. Fifty mosquitoes were put in the space (S) and held in by the perforated piston (P) and the screwed on cap (C). The glass tube (T) was inserted through the 1 in. holes into the cage inside the chamber, a slight movement of the rod (R) pushed the cap (C) away from the tube, and the mosquitoes escaped into the cage.

This apparatus was used for tests on flies and mosquitoes but no attempt was made to obtain a sequence of results and the figures are not given here. Once the apparatus had been completed and found satisfactory, attention was given to working out a method of sampling, and then to using it to obtain data on the behaviour of the insecticide after it had been atomized into the chamber.

TECHNIQUE OF SAMPLING CONCENTRATIONS OF INSECTICIDE IN THE TEST CHAMBER

The first method tried was to suck a measured quantity of the atmosphere in the chamber through a substance chosen to absorb the insecticide carrier. The substance was weighed before and after absorption to obtain the weight of the carrier in the given volume of air. This method usually gave low results, which were probably largely due to the

volatilization of the carrier, the vapour of which was not absorbed, and also to changes in the water vapour content of the absorbent material.

A second method was tried which proved successful. This was to dissolve a dye in the carrier which was then atomized into the chamber; the concentration was sampled when desired by sucking a measured quantity of the atmosphere of the chamber through a sintered glass filter which retained the particles of dye. The retained dye was then washed out of the filter with a measured quantity of undyed carrier and the colour matched with a set of standards in order to ascertain the amount of dye that had been absorbed.

The method is thought to give a good index of the concentration of the insecticide and would also serve to measure the distribution. It is independent of the vaporization of the carrier. With any given carrier it can be used to determine the rate at which the concentration of insecticide falls off and so the relative degree of atomization. The method assumes that the particles of insecticide will behave in a similar manner to the dye particles, and several dyes were tried before the final choice was made. All the dyes behaved in a similar manner, but those chosen were the best for colour comparison. Because the different dye molecules behaved in a similar way, it is reasonable to assume that the insecticide molecules would also behave similarly in this respect.

In addition to being a measure of the concentration of the insecticide itself, the method indicates the behaviour of the carrier after atomization. The percentage of dye remaining suspended is also the percentage of carrier that has not settled out, but remains in the atmosphere either in its original atomized state or as a vapour.

The most satisfactory dyes used were Sudan III for oils, and methylene blue for water. Colour standards were made up in ten glass tubes ($2 \times \frac{3}{8}$ in.). The tubes contained from 0·001 to 0·01 c.c. of the coloured base with sufficient uncoloured oil or water added to make each one up to 3 c.c. The arrangement of the sampling apparatus is shown in Fig. 3.

A Jena sintered glass filter funnel (Büchner type) (GF) of 30 mm. diameter and with pores of 15–40μ was placed inside the cage and connected by glass tubing ($\frac{1}{4}$ in. diameter) to an aspirator (A) outside the chamber.

3 c.c. of the dyed liquid were sprayed in under 30 lb./sq. in. air pressure in a known time and with the fan rotating. After a definite recorded interval (from 10 sec. to 30 min.) the aspirator tap was opened and 2 l. of the atmosphere of the cage were drawn through the sintered

glass filter, which held all the dye particles. The filter had been tested, to ascertain whether the dyes passed through it, by putting a cotton-wool pad beyond it and examining it afterwards for traces of colour.

The dye was washed out with 3 c.c. of spray base into a 2 by ⅜ in. glass tube and the resulting colour compared with the standards.

It was found expedient with the water base experiments using methylene blue as a dye to dilute the standard and wash out the filter with alcohol, as water did not dissolve the dye rapidly enough.

The capacity of the whole chamber was 600 l. and 1/300th of this was removed. That this method of measurement does not result in any significant dilution of the sample by air entering the chamber to replace that which is drawn out is shown by using the equation

$$x = \frac{(ab\,(m-1)-(a-b)\,e^{-m})}{mv},$$

given by Page & Lubatti (1937) in estimating dilution effects on the sampling of fumigants. In this instance v = the volume of the chamber = 600 l.; mv = the volume of the sample = 2 l.; $m = mv/v = 0\cdot003$, a/v = the initial concentration of the atomized material, b/v = the concentration of the atomized material in the outside space, x = the determined concentration of the atomized material in the sample. Since $b = 0$ the equation may be written

$$x = \frac{a\,(1-e^{-m})}{mv},$$

$$\frac{x}{a/v} = \frac{(1-e^{-m})}{m} = \frac{0\cdot0032945}{0\cdot0033333},$$

that is, under the given conditions, the ratio between the actual concentration and the concentration determined for all concentrations of atomized spray inside the chamber = 1/1·01, which, expressed as a percentage, = 98·8 %. Since the colorimetric readings were only accurate to within 10 % it is not necessary to correct for this sampling error due to dilution.

The colour standards contained 0·001, 0·002, 0·003, etc., up to 0·010 c.c. of the coloured spray and the resulting colours given by the aspirated sample therefore corresponded with these standards when the whole chamber contained 300 times this amount of spray, i.e. 0·3, 0·6, 0·9, etc., up to 3 c.c. Therefore standards 1–10 corresponded to 10, 20, 30, etc., up to 100 % of the 3 c.c. originally sprayed in.

For more accurate measurements a colorimeter would be necessary,

but it was found possible to judge within 10% with certainty with the method described.

Some experiments were done using White May kerosene, the specification of which has already been given: and some with Shell oil 24210, a white oil of the following specification: sp. gr. = 0·862, flash-point (closed) 320° F., flash-point (open) 335° F., visc. redw. 1 at 70° F. = 118 sec., pour test = − 30° F., unsulphonated residue = 99·2 % by volume and sufficiently refined to be odourless and nearly tasteless; and water.

Table II.   *The rate of settling out of a light oil (White May kerosene)*
stained with Sudan III. Each of series 1–6 shows the rate of settling out
at a different degree of atomization as indicated by the time taken to spray
3 c.c. in at a given pressure

Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in	Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in
Series 1: 3 c.c. of oil atomized in 4½ sec.			Series 2: 3 c.c. of oil atomized in 6½ sec.		
6 sec.	1·65	55	10 sec.	2·1	70
30 sec.	1·13	38	30 sec.	1·75	58
60 sec.	1·05	35	—	—	—
2 min.	0·92	31	2 min.	1·10	37
5 min.	0·62	21	5 min.	0·87	29
10 min.	0·45	15	10 min.	0·76	25
20 min.	0·35	11	20 min.	0·54	18
Series 3: 3 c.c. of oil atomized in 10 sec.			Series 4: 3 c.c. of oil atomized in 13½ sec.		
7 sec.	2·5	83	9 sec.	2·76	92
30 sec.	2·4	80	30 sec.	2·73	91
60 sec.	2·2	73	60 sec.	2·60	87
2 min.	1·9	63	2 min.	2·30	77
5 min.	1·6	53	5 min.	1·70	57
10 min.	1·2	40	10 min.	1·30	43
20 min.	0·95	32	20 min.	1·05	35
Series 5: 3 c.c. of oil atomized in 20 sec.			Series 6: 3 c.c. of oil atomized in 65 sec.		
9 sec.	2·76	92	7 sec.	2·79	93
—	—	—	—	—	—
60 sec.	2·72	91	60 sec.	2·73	91
2 min.	2·67	89	2 min.	2·70	90
—	—	—	5 min.	2·55	85
10 min.	2·16	72	10 min.	2·40	80
20 min.	1·73	58	20 min.	2·10	70

Table II and Fig. 5 show how dye particles carried in White May kerosene settle out at different degrees of atomization. The degree of atomization was adjusted by keeping the air pressure constant, but adjusting the liquid needle valve so that the 3 c.c. went out at different speeds. The faster the liquid went out the coarser the degree of atomization. It is evident that the degree of atomization has a very marked effect on the amount of material remaining suspended in the atmosphere. With the comparatively coarse atomization of series 1 and 2 there is an initial
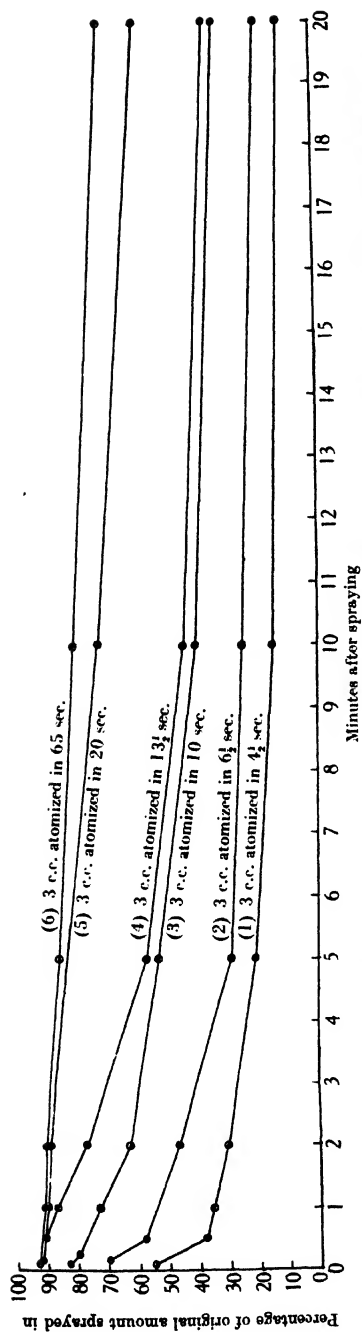
Fig. 5. Graph showing the rate of settling out of the colour when a light oil (White May kerosene) stained with Sudan III is atomized into a chamber. Each of series 1–6 shows the rate of settling out at a different degree of atomization. See Table II.
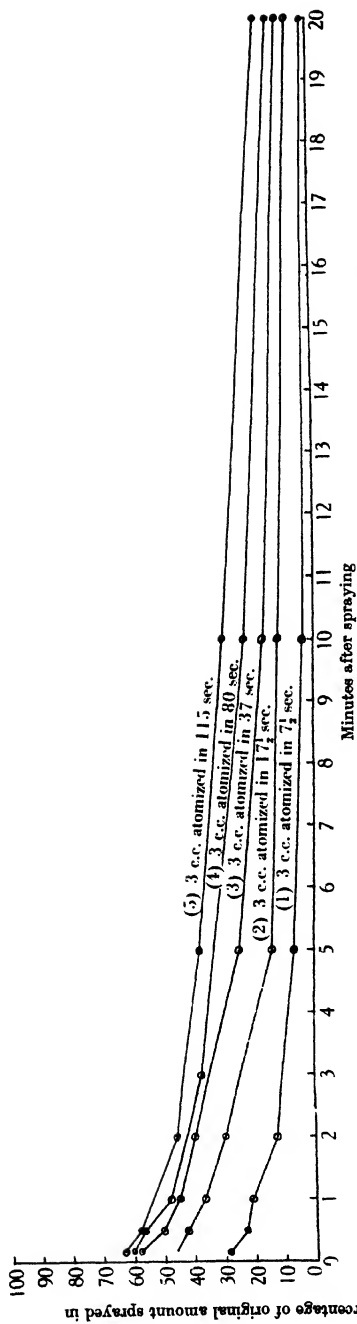
Fig. 6. Graph showing the rate of settling out of the colour when a white oil (Shell oil 24210) stained with Sudan III is atomized into a chamber. Each of series 1–5 shows the rate of settling out at a different degree of atomization. See Table III.

rapid fall in concentration, probably produced by the larger droplets settling out, followed by a steady slower drop. In series 3–6 this initial drop is not so marked and a high proportion of the material is still suspended at the end of 2 min. The proportion at the end of 20 min. increases with increasing fineness of atomization. From the point of view of the testing of insecticides these figures show that consistent and comparable results cannot be expected unless the degree of atomization is standardized either by sampling or some other method. They also show that unless the insecticide is finely atomized, the time at which the insects are introduced is important, because during the first 30 sec. the concentration is falling rapidly and unless the insects are present throughout the spraying, or are introduced at exactly the same time on every occasion, or are introduced after the first 30 sec., the concentrations to which they are exposed will be very different.

Table III. *The rate of settling out of the white oil (Shell oil 24210) stained with Sudan III. Each of series 1–5 shows the rate of settling out at a different degree of atomization as indicated by the time taken to spray 3 c.c. in at a given pressure*

Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in	Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in
Series 1: 3 c.c. of oil atomized in 7½ sec.			Series 2: 3 c.c. of oil atomized in 17½ sec.		
10 sec.	0·87	29	10 sec.	1·37	46
30 sec.	0·70	23	30 sec.	1·26	42
60 sec.	0·62	21	60 sec.	1·10	37
2 min.	0·40	13	2 min.	0·90	30
5 min.	0·22	7	5 min.	0·43	14
10 min.	0·11	4	10 min.	0·35	12
20 min.	0·06	2	20 min.	0·22	7
Series 3: 3 c.c. of oil atomized in 37 sec.			Series 4: 3 c.c. of oil atomized in 80 sec.		
10 sec.	1·75	58	10 sec.	1·80	60
30 sec.	1·50	50	30 sec.	1·70	57
60 sec.	1·35	45	60 sec.	1·45	48
2 min.	1·20	40	2 min.	—	—
5 min.	0·76	25	3 min.	1·10	37
10 min.	0·50	17	10 min.	0·70	23
20 min.	0·30	10	20 min.	0·40	13
Series 5: 3 c.c. of oil atomized in 115 sec.					
10 sec.	1·88	63			
30 sec.	1·73	58			
60 sec.	—	—			
2 min.	1·37	46			
5 min.	1·15	38			
10 min.	0·90	30			
20 min.	0·50	17			

Table III and Fig. 6 show the results of an experiment with Shell oil 24210 similar to the above. The same general results are illustrated in this experiment as in the preceding one.

The most important additional point that this experiment demonstrates is the difficulty of comparing insecticides in different carriers. A dye in White May kerosene sprayed in $6\frac{1}{2}$ sec. remains suspended better than the same dye in Shell oil 24210 sprayed in 115 sec. under the same conditions. From these results it is clear that where insecticide carriers of different physical properties are being used, it is not possible to make a fair comparison of the toxicity unless some method such as sampling is adopted to ensure that equivalent amounts of the toxic material remain in suspension.

This experiment also shows that the heavy oil is more difficult to atomize satisfactorily. In order to break up the particles sufficiently finely within a reasonable space of time it would be necessary to use a higher air pressure than that used in these experiments.

Table IV. *The rate of settling out of water stained with methylene blue in an unsaturated atmosphere. Each of series 1–4 shows the rate of settling out at a different degree of atomization as indicated by the time taken to spray 3 c.c. in at a given pressure*

Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in	Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in
Series 1: 3 c.c. of water atomized in 5 sec.			Series 2: 3 c.c. of water atomized in 10 sec.		
10 sec.	1·2	40	10 sec.	2·05	68
20 sec.	1·05	35	60 sec.	1·95	65
2 min.	1·0	33	2 min.	1·86	62
5 min.	0·95	31·5	5 min.	1·70	56·5
10 min.	0·84	28	10 min.	1·75	58
20 min.	0·80	26·5	20 min.	1·70	56·5
Series 3: 3 c.c. of water atomized in 23 sec.			Series 4: 3 c.c. of water atomized in 57 sec.		
10 sec.	2·5	83	10 sec.	2·92	97
60 sec.	2·0	66·5	60 sec.	2·86	95
2 min.	1·95	65	2 min.	2·60	86·5
5 min.	– –	—	5 min.	2·40	80
10 min.	1·95	65	10 min.	2·0	66·5
20 min.	1·75	58	20 min.	1·97	65·5

The results of the experiments using a water base stained with methylene blue are presented in Tables IV and V and Figs. 7 and 8. Both these sets of results confirm the general conclusions of the previous experiments. They also show that when a water base is used the amount remaining suspended in the atmosphere under given conditions of atomization depends on the initial degree of saturation of the atmosphere as well as on the degree of atomization. The material settles out far more rapidly in a saturated atmosphere than in an unsaturated one.

The primary object of the chamber method of testing and comparing the toxicity of insecticides to flying insects is to expose the insects to a
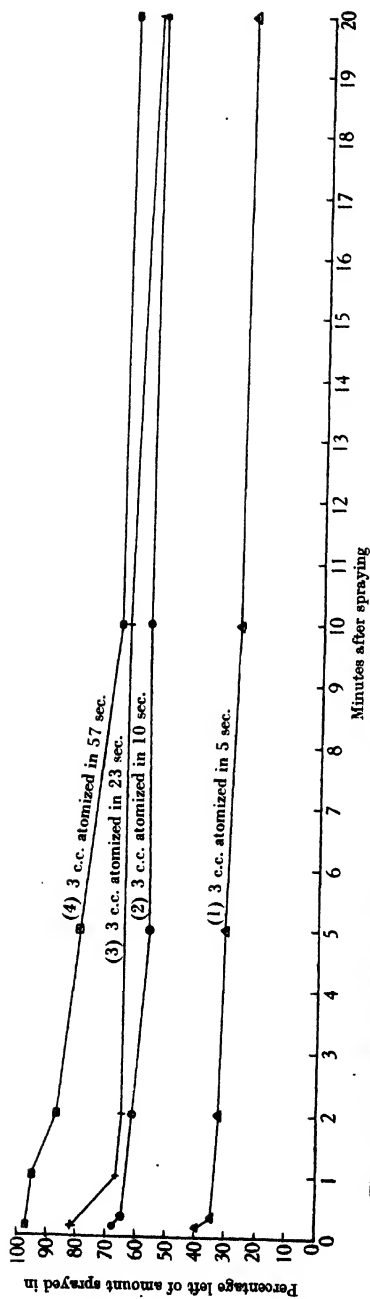
Fig. 7. Graph showing the rate of settling out of the colour when water stained with methylene blue is atomized into an unsaturated atmosphere. Each of series 1–4 shows the rate of settling out at different degrees of atomization. See Table IV.
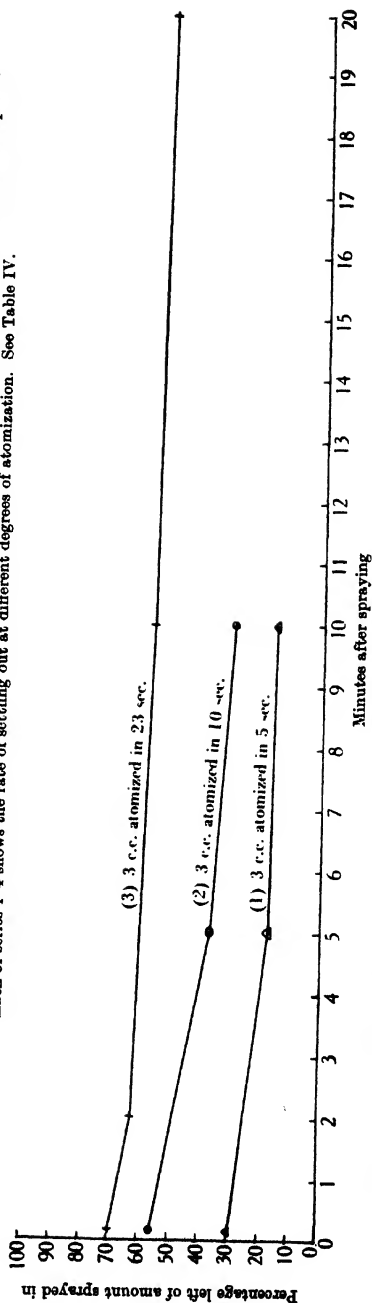
Fig. 8. Graph showing the rate of settling out of the colour when water stained with methylene blue is atomized into a saturated atmosphere. Each of series 1–3 shows the rate of settling out at different degrees of atomization. See Table V.

Table V. *The rate of settling out of water stained with methylene blue in a saturated atmosphere. Each of series 1–3 shows the rate of settling out at a different degree of atomization as indicated by the time taken to spray 3 c.c. in at a given pressure*

Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in	Interval before sampling	Conc. remaining in air c.c.	% of amount sprayed in
Series 1: 3 c.c. of water atomized in 5 sec.			Series 2: 3 c.c. of water atomized in 10 sec.		
10 sec.	0·90	30	10 sec.	1·70	56·5
5 min.	0·54	18	5 min.	1·10	36·5
10 min.	0·48	16	10 min.	0·90	30
Series 3: 3 c.c. of water atomized in 23 sec.					
10 sec.	2·10	70			
2 min.	1·90	63			
10 min.	1·72	57			
20 min.	1·60	53			

given quantity of the material suspended in the atmosphere of the chamber under standard conditions. These experiments show that it is difficult if not impossible to do this continuously unless a sampling method is adopted. If the same carrier is utilized throughout, a slight change in the atomizer may alter considerably the amount remaining suspended after a given time. Unless the insects are kept in the chamber throughout the spraying, which has the disadvantage that they are exposed to an unequally distributed insecticide, it is desirable to know when the initial rapid fall in the insecticide has taken place in order that the insects may ' be introduced after it has occurred. If different carriers are being used it is essential when making comparisons that the behaviour of each carrier after it has been atomized should be known.

The sampling methods described above should make it possible to obtain considerably more information than is at present available on the behaviour of atomized insecticides, always providing that the dye molecules behave in a similar manner to the insecticide molecules.

SUMMARY

1. An apparatus and method for testing the effect of atomized sprays on flies and mosquitoes are described. The apparatus consists of a revolving wire gauze cage placed in a thermostatically controlled chamber, the whole of which may be easily cleaned and freed from toxic residues. The insecticide is sprayed into the chamber by means of an Aerograph MP gun and distributed by means of a slow-moving fan. When the insecticide has been injected and an interval allowed for the initial rapid fall of concentration, the movement of the cage is stopped and the

insects are introduced into it by means of a special tube and plunger. The time required for paralysis to take place is recorded. After a given interval the insects are removed from the gauze cage and kept to ascertain the mortality.

2. A technique for sampling the concentration of insecticide in the air space is described. The insecticide carrier is coloured with a dye, Sudan III for petroleum oil bases and methylene blue for water bases. The percentage of atomized material remaining in the atmosphere at any given time is determined by aspirating a known quantity of the atmosphere of the chamber through a sintered glass filter. The dye is retained in the filter, it is washed out with a measured quantity of liquid and compared with known standards. Reasons are given for the assumption that the dye molecules will behave in the same way as the insecticide molecules.

This sampling method has been used to study the behaviour of a light oil, a heavy white oil and water at different degrees of atomization. Tables and graphs are given which show that, except with a fine atomization where most of the insecticide remains suspended for a considerable time, there is an initial rapid fall, which varies in amount with the degree of atomization. This initial fall is followed by a much more gradual decrease of concentration. The experiments show clearly that oil bases of different physical properties cannot be compared adequately unless a sampling method is used to ascertain the quantities of material remaining suspended. Where water bases are used it is shown that the time concentration curve varies not only with the degree of atomization, but also with the degree of saturation of the atmosphere before spraying. The concentration remains higher in an unsaturated atmosphere than in a saturated one.

We are indebted to Prof. J. W. Munro, in whose department the work was carried out, and to Dr A. B. P. Page of this department for many helpful suggestions on the method of sampling.

REFERENCES

PAGE, A. B. P. & LUBATTI, O. F. (1937). Determination of fumigants. VIII. Sampling from small spaces. *J. Soc. chem. Ind.* **56**, 54–61.

PEET, C. H. (1932). The Peet-Grady method revised. *Soap*, **8**, no. 4, 98–102, 121.

PEET, C. H. & GRADY, A. G. (1928). Studies in insecticidal activity: testing insecticides against flies. *J. econ. Ent.* **21**, 612–17.

RICHARDSON, H. H. (1931). Insecticidal method for the estimation of kerosene extracts of pyrethrum. *J. econ. Ent.* **24**, 97–105.

BIOLOGICAL METHODS OF TESTING INSECTICIDES

A REVIEW

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CONTENTS

INTRODUCTION

INSECTICIDES can be divided into three classes: (*a*) contact insecticides, (*b*) stomach poisons, (*c*) fumigants. Those in class (*a*) act by penetration of the integument and tracheal system; in (*b*) by ingestion in the intestinal tract; (*c*) probably react in approximately the same way as the contact insecticides, but through the vapour phase and by a readier access to the tracheal system. The three classes require different methods for their biological evaluation both in the laboratory and the field.

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LABORATORY METHODS

(a) Contact insecticides

These can be divided into the following classes according as the technique involves
(i) spraying, (ii) dipping, (iii) the use of the micropipette, (iv) dusting.

Spraying technique.

The methods, involving the use of finely atomized spray fluids, are of two kinds:
those in which the liquid is thrown in the form of fine droplets on to the insects on a
surface or flying about in a chamber. The latter is usually employed only in work
upon household sprays, and houseflies are almost universally employed as test subjects.

Insects on a surface. Tattersfield & Morris (1924) elaborated an apparatus, later
modified with respect to its atomizer (Tattersfield, 1934), so arranged that successive
batches of insects on a surface could be sprayed under controlled conditions with
respect to pressure of spray and quantity of insecticide atomized upon the insects.
It consisted of a glass jar with external and internal levelling platforms; upon the
latter a small glass dish containing the insects could be placed at a fixed distance from
the atomizer, held in the lid by a clamp. The internal platform stood on levelling
screws on a glass disk reposing on a small glass tripod and the atomizer was so
arranged that the cone of spray could be adjusted to fall as evenly as possible about
the dish. The dish of insects was placed in the instrument by removing the lid, and the
fluid, already pipetted into a small reservoir, was immediately atomized by means of an
air-line connected through a pressure gauge to a cylinder of compressed air fitted with
an adjusting valve. Later models were of square section and the insects were intro-
duced through a sliding door in one of the sides. This form of apparatus has been used
by Steer (1938). Small sucking insects, e.g. aphides (Tattersfield & Morris, 1924), or
insect eggs (Gimingham *et al.* 1926) can be used as test subjects. One of the main
advantages of the apparatus consists in the small amount of spray fluid required; it
can, however, be criticized on the grounds that the area, over which the deposit of
spray is relatively evenly distributed, is small.

Potter (1938, private communication) has constructed a spray apparatus which,
although of a similar type to that of Tattersfield & Morris, achieves a greatly improved
distribution of the spray fluid over a larger area. It involves the use of a larger amount
of spray fluid, and the employment of a mixing tower above the spray chamber. The
cone of spray is by this means broken up, and the droplets fall in an evenly dispersed
shower over the area upon which the insects are placed.

O'Kane *et al.* (1930), in an important paper on the factors involved in the per-
formance of contact insecticides, described a slightly different type of apparatus, in
which the insecticide was sprayed diagonally upon the insects placed upon a rotating
disk.

Jones *et al.* (1935) modified these methods with the object of saving time and
labour and to obtain greater uniformity of application. The apparatus evolved is now
widely known as the Campbell turn-table. Glass cylinders on a turn-table can be
moved successively under a spray-gun, the insecticide is sprayed in, the cylinder
allowed to stand for a short but definite length of time and then moved laterally over
house-flies, just recovering from quiescence induced by chilling or anaesthesia, in a
dish covered by wire mesh. In all these methods the effect of the insecticide is judged
after certain periods of time, the numbers of insects affected are placed in categories,
and the toxic effect produced correlated with the concentration.

Since Campbell's method depends on the deposition on the insect of a falling mist of insecticide, the larger droplets having been got rid of by the initial standing, certain very definite precautions are requisite if results are to be replicated. As first described, the jar with the enclosed mist was moved by hand over the insects; in order that the weight of deposit should be the same in repeat experiments, the time taken and the procedure adopted had to be reproduced with considerable care. Moreover, the use of wire mesh over the insects may give rise to complex eddy currents, and the employment of chilled or anaesthetized flies may introduce factors the effects of which upon insect resistance are imperfectly known. They are perhaps to be deprecated. Zermuehlen & Allen (1936) modified the process by using a removable paper cover over the insect container; this was withdrawn and the container pushed into the jar containing the insecticide mist. Campbell & Sullivan (1938) have recently elaborated an improved turn-table method, and as a result of their investigation have made further suggestions directed towards the improvement, simplification and cheapening of the apparatus. It is constructed entirely of metal, consisting of a triangular frame on which is mounted a circular aluminium turn-table (46 in. diameter). The movable glass cylinders are replaced by aluminium ones (17 in. high and 8 in. diameter), ten in number. Below each of these are cups (4 in. deep and 6 in. diameter) attached to the underside of the table. These hold the cages of insects which can be cut off from the upper cylinder by means of stainless steel slides running in slots in the table. When operating for testing fly-sprays a $5\frac{1}{2}$ in. screen-covered Petri dish containing 100 flies is placed in one of the cage-holders, the slide pushed in to cover it and the larger aluminium cylinders placed over it. A known volume of the appropriate spray fluid to be tested is atomized at constant pressure into the upper cylinder through a hole in the lid, the larger droplets are allowed to settle for a known time, the slide is withdrawn and the fine mist allowed to deposit upon the flies in the cage below. After 10 min. exposure, the Petri dish is removed and the treated flies transferred to observation cages. By turning the table and bringing the spray cylinders successively into action, a number of tests can be carried out with considerable rapidity. The method was primarily designed for testing fly-sprays for which an oil base is used. Badertscher (1936) compared the turn-table method with the standard Peet-Grady technique (see below) and found that he obtained higher percentage kills with the Peet-Grady apparatus for pyrethrum and thiocyanate sprays, a result confirmed by Campbell & Sullivan (1938). Badertscher obtained, however, the curious and apparently anomalous result that the reverse was the case for sprays containing rotenone. Speed in operation and the fact that aqueous sprays can be tested are stressed as advantages of the turn-table method. The method depends upon the settlement of a mist, and as has been pointed out by Campbell & Sullivan (1938) it is possible that, for insects above a certain level of resistance, the maximum deposit of liquid available may be insufficient to secure a satisfactory kill. The authors suggest that this difficulty might be met by spraying directly through the cage containing the insects by removing the disk on the bottom of the cage-holder.

If reliable and replicable results are to be obtained by the use of this apparatus, there is no question that the whole sequence of operations must be carried out in as uniform a way as possible. Not only so, but the insects used should be reared as uniformly as possible.

Other methods depend upon spraying infested plants while they are being slowly

rotated. Hartzell & Wilcoxon (1932) employed such a technique. Immediately after spraying, the plants (nasturtiums) are placed with the pot lying on its side, so that the plant itself lies over squared paper surrounded by a barrier of grease or gummy material. The effect produced was determined by examination under a binocular microscope with probing—any insect showing movement was regarded as alive.

Lethal-chamber methods. These are chiefly used for testing fly-sprays. The Peet-Grady method (1928, 1932) was one of the earliest to be so employed. Essentially the apparatus is a small room, $6 \times 6 \times 6$ ft. inside, constructed of non-absorbent walls with impermeable sealed corners. It is fitted with a tightly flush-fitting entrance door and observation windows in the centre of each wall, the ceiling has a window 18 in. square, 6 in. above which a 200-watt light bulb is set for illumination. In each lower corner a 6×6 in. square port is cut, covered by wire gauze and tight-fitting hatches.[1] Two 1 in. holes are bored in each wall 6 in. from the ceiling, 1 ft. from the edge, which can be closed by corks. A fan and wire-covered hatch for changing the air after each test are fitted opposite the entrance. The technique for operating involves the liberation of flies, of a definite age and reared under as constant a set of conditions as possible, into the chamber and the subsequent spraying, under a definite pressure, of a total of 12 c.c. of the insecticide solution in about equal amounts through the eight 1 in. holes. After 10 min. the ventilating fan is started, after opening the screen ports, and the flies carefully taken out, counted, put into observation cages and kept under a relatively uniform set of conditions. The total number of flies disabled after 10 min. is calculated to the percentage used and termed the "knock-down" percentage, but the insecticide is rated by the percentage apparently dead after 24 hr.

The Peet-Grady Test was adopted in 1932 by the American National Association of Insecticide and Disinfectant Manufacturers as the standard method for testing fly sprays. It was early noted that, whereas a given laboratory could reproduce its evaluations with fair accuracy, there was considerable difference between the results of different laboratories. A conjoint investigation was undertaken to ascertain the measure of agreement, and, to find out if the discrepancies could be overcome by employing a common standard insecticide, in the first instance benzophenone, for purposes of comparison. Campbell (1938) analysed the results obtained in this co-operative effort. It is not a matter of surprise that large discrepancies should have resulted when the comparative evaluations were based on the average percentage of flies killed, as the technique of testing and the methods of rearing the flies, and to some extent the equipment, varied from laboratory to laboratory. Nor did the standard insecticide proposed help in clearing up the difficulties; it was finally discarded on Campbell's suggestion for one prepared from pyrethrum. Campbell considered that efforts should be made to reduce variations of results within laboratories as much as possible, but he considered that a mode of comparative assessment and of expressing results proposed by Simanton (1937) was a feasible one. This involves ten paired tests of the standard and of the insecticide to be compared with it. In the trials the standard insecticide should give between 30–70% mortalities, if possible between 50–60%. In a series of randomized tests the mean difference between the kill obtained with the unknown insecticide and the standard should be determined, together with the

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[1] Through one of these the flies are introduced.

standard error of the mean of ten differences as a measure of variability; if the latter value is less than 3 the test is regarded as having been properly conducted. The mean difference gives the grade. These proposals have been accepted by the National Association of Insecticide and Disinfectant Manufacturers of the United States, who have issued in considerable detail the procedure to be adopted in rearing the test flies and for carrying out the tests, assessing and reporting the results (see *Soap Blue Book*, 1938, pp. 145, 147, 153).

H. H. Richardson (1931) also describes a chamber method. He uses a rectangular box (2¼ × 2¼ × 4 ft.) with glass top and sides, in which a movable copper wire screen cage (1½ × 2¼ × 1½ ft.) is placed. He incorporates a temperature control and also a fan opposite the atomizer, which is centrally fixed and projects into the test box, so that the spray is atomized into the cage, which is open at the bottom and standing on heavy wrapping paper. The fan is switched on, 40–60 flies reared under constant conditions introduced into the cage and the spray fluid (1·6 c.c.) atomized. At 30 sec. intervals, counts of the number of paralysed flies are made until well over 50% of the flies are down. After 8 min., the flies are counted and transferred to a special cage. The speed of paralytic action is taken as the time required to paralyse 50% of the flies, and it was found in the case of kerosene-pyrethrum sprays that this varied directly with their strength and was a very sensitive record of it. The death curve, obtained by plotting the percentage of the insects killed in 24 hr. against the concentration, was less sensitive.

Potter & Hocking (this number, p. 348), have studied the concentration of oil insecticides in a spray chamber, of a type similar to that of Richardson, in successive intervals of time. They find that it depends upon the rate at which the fluid is atomized into the chamber, the slower the rate at a given pressure the higher the concentration, and that it falls off rapidly at the beginning and more and more slowly with time. They also find that, with a given rate and pressure of atomization, the initial concentration is higher with the lighter than with the heavier oils, owing to the formation of smaller droplets, but other effects, such as evaporation, affect the concentration of the insecticide.

Lethal-chamber methods, although corresponding more closely than any other with actual large-scale conditions in so far as fly-sprays are concerned, are open to the objection that little is known about the distribution of either the insecticide droplets or of the insects inside the chamber. It would appear to be necessary to run a series of tests with a standard insecticide along with those of the unknown, if the results are to be susceptible of quantitative interpretation. They are also only available for such flying insects as houseflies.

Campbell & Sullivan (1938), Richardson (1932), Simanton & Miller (1938) have laid down in some detail the methods they employed for rearing and handling house-flies for spraying purposes. The age of the insect used is of importance, but Simanton & Miller (1937) have shown that with houseflies there is little difference in suscepti-bility between 4, 5 and 6 days old insects. Tuma (1938) has shown that resistance of cockroaches (*B. germanica*) to pyrethrum and aliphatic thiocyanates increases up to an age of 17 weeks, after which a decline follows. Other factors of importance may be length of time elapsing between feeding and spraying, the degree of crowding of a culture and the amount and intensity of illumination. Further, Murray (1937, 1938) and Miller & Simanton (1938) have demonstrated the greater susceptibilities of the

male over female houseflies to sprays and the importance of the determination of the sex ratio of the samples used in the tests. The readiest way to overcome the difficulty is to obtain 50% approximately of each sex by preserving the inherent sex ratio of the reared cultures and using all the flies in each cage as a test unit.

Dipping methods.

These methods have been used over a considerable time; for example, Fryer *et al.* (1923) employed this technique in their investigations of derris as an insecticide. Shepard & Richardson (1931) described a dipping method which they used for testing nicotine against *Aphis rumicis*. They determined toxicity curves for the two alternatives, (*a*) where the concentration is variable and time of immersion constant and (*b*) where time of immersion is variable and concentration constant. They considered that, since in their method a wetter which might have a toxic action was not used, it had advantages over spraying technique where adequate wetting must be secured. Fleming & Baker (1934) also preferred a dipping technique for their work on the effect of contact insecticides on the Japanese beetle (*Popillia japonica*). The coefficient of effectiveness was obtained by dividing the median lethal concentration, after submersion for 120 sec., of the standard by that of the test material. A dipping method for testing egg-killing washes is also described by Kearns & Martin (1936) and by Steer (1938).

A careful study of this technique as applied to adult insects and their reaction to derris preparations has been made by Craufurd-Benson (1938) in which he examines the various factors involved, such as the age of the insects, the temperature and humidity conditions under which they are reared, the period of starvation before dipping, the time of immersion and the temperature of the bath. He evolved an improved immersion method which gives reproducible results of considerable accuracy with the particular test insect chosen. His apparatus consists essentially of a copper water-jacketed thermostat of a special type, the inner bath of which is kept at constant temperature by an electric immersion heater and stirred with an electrically driven paddle. The insecticide liquid held in a small glass beaker is placed in the inner bath until the temperature required is reached, and the insects in muslin-capped tubes are dipped in it for a definite period of time. After dipping, the tube is drained and dried by a constant procedure and the insects set aside in specimen tubes to be examined after 24 hr. The percentage numbers of insects killed for each concentration are determined. The conditions in Craufurd-Benson's experiments were: (*a*) insect, *Ahasverus advena*, (*b*) that the age of the insect should be 10–20 days, (*c*) food, mouldy rolled oats and dried yeast, (*d*) the temperature throughout breeding, and before, during and after the experiment, 25° C., (*e*) the humidity, 75% R.H., (*f*) immersion period, 4 min., (*g*) starvation period of insect, 24 hr., (*h*) observation period, 24 hr.

The most valid objection to the use of dipping methods appears to be that there is a risk of stomach poison effects being added to those given by contact. Craufurd-Benson considers this risk outweighed by other advantages.

Micropipette drop method.

This method is as far as possible free from the last objection. O'Kane *et al.* (1933) applied droplets, weighing 2 to 3 mg. each, to various areas of the last instar of the mealworm (*Tenebrio molitor*). Nelson *et al.* (1934) elaborated the method. Sixty or seventy

house flies are cooled in an electric refrigerator at $-1°C$. (30° F.) until quiescent. A certain number are selected for uniformity of size and age, turned on their backs on a marble slab, 0·75 mm.[3] of an alcoholic solution of the insecticide (or 9 parts ethyl alcohol plus 1 part of the liquid insecticide) is placed by means of a capillary pipette on the centre of the ventral surface of the thorax of each of the insects, while still inactive. They are afterwards placed in covered dishes with a supply of food and observed after 24 hr. They are then listed in categories of active, moribund and dead. The results given by different concentrations can be compared with those given by a standard insecticide. The flies should be reared under a constant set of conditions and preferably separated by sex after chilling. Accuracy, small amount and cost of equipment and adaptability of the method to other insects are claimed as advantages; but the authors state its disadvantages to be, that it is not comparable with field conditions and requires a considerable degree of skill.

Dusting methods.

The methods used for testing the contact insecticidal values for dusts have not been worked out in such great detail as those for testing liquid contact sprays or for stomach-poison dusts. The difficulties involved are (a) the difference in the rate of deposition and in the distribution of particles of different size, (b) difficulty in dilution, (c) the possibility of the separation of the diluent by air-flotation, a difficulty which may possibly be overcome in the case of vegetable insecticides by the use of organic diluents (e.g. powdered walnut shell), (d) the aggregation of particles during the procedure of dusting.

Trappmann & Nitsche (1934) and Thalenhorst (1937) have adopted and simplified a method of Gornitz (1933). Thalenhorst used a glass bell-jar placed over a box with sliding lid. The box contained a thick sheet of paper (area 150 sq. cm.) resting on a scale-pan, attached by a rod through a slit in the box side to one arm of a balance. The larvae to be dusted were placed in dishes in the box. With the lid closed, dust was blown into the bell-jar, the lid was then opened, the dust settled on the paper and on the larvae. When a predetermined amount of dust had fallen on the paper the lid was closed and the larvae taken out and placed on cabbage leaves. Care as to breeding and size of the insects has to be taken if accurate and reproducible measurements are to be made.

(b) Stomach poisons

In the case of contact insecticides it is assumed, if the spray or dust is evenly distributed, that the amount coming into contact with insect test-subjects of the same species and stage of development is proportional to their size, and that the amount absorbed is proportional to the concentration of the poison. For stomach poisons special precautions have to be taken if the effect produced is to be correlated with the dose administered. Much recent work has been devoted to securing this end. Special difficulties are met with when, as in the case of vegetable poisons, e.g. nicotine and derris, the material has both a contact and stomach-poison effect, and methods available for testing mineral poisons such as the arsenates might give inaccurate and misleading results unless special precautions were taken. Thus Janisch (1926) proposed feeding the larvae of *Pieris brassicae* on leaves weighed before and after dusting, the outlines of which were traced on squared paper before and after feeding. In this way the amount of leaf and poison consumed could be determined. This pioneer method

would fail to separate the contact and stomach-poison effects if the insect were allowed to move over the surface of the dusted leaf, and the same criticism would also be valid for many methods involving the use of poisoned baits. The toxicity data obtained would represent the total insecticidal effects, but there would be no means of quantitatively assessing the significance of each of them. In the case of nicotine, respiratory toxic action in the gaseous phase, in addition, might well play an important part. The problem, therefore, of differentiating the two effects is not an easy one; injection methods are too remote from practical utility and too slow in operation to permit of ready use for assessment purposes, and the ruling out of some contact action at the mouth parts seems not only impracticable but an unnecessary refinement for anything but critical physiological work.

Drop method.

Price (1920) administered known quantities of arsenical solutions to individual insects; Campbell (1926 *a*, *c*) developed and refined the technique to make it one of considerable precision. In his earlier experiments (1926 *c*) he placed a drop, containing a known concentration of poison, from a weighing burette on a leaf surface in the feeding path of the caterpillar (previously weighed); when the poison was consumed, the burette was reweighed. This procedure was repeated with other caterpillars and he determined the survival time (hence its reciprocal "the speed of toxic action") for the weight of poison consumed per gram body weight of insect. He was thus able to ascertain the relative potencies of arsenite to arsenate, and to show that insects differ markedly in their susceptibility to arsenical poisons. The method would appear too tedious and laborious for general application and later Campbell (1926 *a*, *b*) designed a microburette technique by means of which measured droplets could be placed upon the mouth parts of silkworms of known weight. The speed of toxic action for each concentration of poison administered was determined. Goetze (1932) also developed a micropipette method for comparing pyrethrum with the arsenicals. The poisons were diluted with honey solution (2 : 1), taken up in the graduated pipette, which was laid on its side. The tip of the pipette passed through a hole of a cage. Bees were used as test insects, the amount taken being measured and the toxic effect recorded.

Leaf-sandwich method.

Another technique, known as the leaf-sandwich method, was devised by Campbell & Filmer (1929); it is a great improvement on Janisch's leaf method. It is primarily applicable to dusts, but there is no obvious reason why it should not be adapted to spray fluids. Essentially, the technique consists of blowing a quantity of the poison dust into an inverted bell-jar through a tube fitted with a ball-valve. After a short period, during which the larger particles are deposited, the jar is moved on to a plate on which are distributed circles of leaf and a number of circular glass cover-slips of known area. The cover-slips are weighed to determine the amount of the deposit, the leaf circles are made into sandwiches by superimposing other leaf circles of the same size and are fed to individual caterpillars, the weights of which are known. After feeding, the unconsumed area is determined either by means of a planimeter, cross-section paper or by photoelectric cell (Bulger, 1935). Stellwaag (1931) suggested some simplification of the process and coated the leaf disks with starch paste. Görnitz (1933) introduced a method for directly weighing the deposit, by means of a frame inserted into the dusting chamber and attached by means of a bar to one arm of a balance;

thus predetermined weights of poison can be deposited. Bulger (1937) has also suggested means for controlling the amount of deposit consumed.

By this method much important toxicological data can be accumulated. The speed of paralytic action (100 divided by the active period in hours) and the speed of toxic action (100 divided by the survival time) can be determined for each dose consumed. In addition, the proportional number of deaths can be ascertained for the amounts of poison consumed. The latter values enable a table to be constructed indicating the sublethal, intermediate and lethal doses. The chief defect, according to Campbell & Filmer (1929), of any leaf-area method is that the doses cannot be accurately predetermined and they consider that the numbers of larvae in the sublethal and lethal zones are wasted, and that time and insects are thus thrown away, since they do not help in delimiting the medium lethal dose (that dose which kills 50%). This dose occurs in the intermediate zone of toxicity and can be approximately determined from a consideration of the data accumulated within that zone. The modifications proposed by Bulger (1937), by Görnitz (1933) and by Thalenhorst (1937) may have eliminated some of the waste of insects.[1]

Campbell (1930), however, has pointed out that the median lethal dose does not present a complete picture of the effectiveness of a stomach poison, since the time factor involved in death or recovery is unstated. Indeed, Campbell & Filmer (1929) consider that the effectiveness of a stomach poison should be clearly differentiated from toxicity; the latter term implies killing power, while the former, in addition, is influenced by the rate or speed of toxic action and by the cumulative deterrent effects upon further feeding. Campbell (1930) illustrated the necessity of close attention to the factors determining relative toxicities by plotting against the dosage (mg. per g. body weight) the speed of toxic action, as represented by the reciprocal of the active period and as represented by the reciprocal of the survival period, for two different poisons (sodium silicofluoride and acid lead arsenate). He determined the areas between each curve, the ratio of which gives the relative toxicity over the whole dosage range. He pointed out that, since upon the onset of paralysis no further feeding takes place, the determination of the active period of the insect may be a better assessment of relative toxicity than that of the survival period. In such a case the terms relative toxicity and relative effectiveness would appear to merge into one meaning.

Bulger (1937) has pointed out that the median lethal dose of an insecticide dust may depend partly on the size of particle. In the sandwich method only the smaller particles are utilized. It would thus appear that, for purposes of determining the relative potencies of more than one sample, the particle size should be of comparable dimensions, a requirement which, for compounds of widely different density, may not be easy to attain.

Cage method.

This method, in which several insects are confined in each cage with treated foliage, is not, as generally used, capable of giving an evaluation of the stomach-poison action alone. It affords an indication of total toxic action and deterrency. It was used by Gimingham & Tattersfield (1928) for some preliminary work on the reaction of

[1] Bulger (1932) mentions another difficulty inherent in the method, namely, the possible separation of diluent or adjuvant from the poison in the process of settling, necessitating separate applications of the components.

mandibulate insects to foliage sprayed with extracts of certain leguminous fish-poisons, and is the only method available in special cases, such as that of exploring the toxic action of chemicals to shy-feeders such as the Japanese beetle (*Popillia japonica*), for which specially constructed and illuminated cages were used by Fleming (1934). He demonstrated the great importance of the effect of temperature, humidity and degree of illumination on the susceptibility of this insect.

Plug method.

This method is primarily used for testing insecticides against codling-moth larvae, and by its nature it measures the total toxic effect due to both stomach and contact action, if the latter is shown by the compound. Newcomer (1926) sprayed apples upon which newly hatched larvae were placed and the number of worm entrance-holes and stings determined. Siegler & Munger (1933) improved this technique by taking cylindrical plugs of de-cored apples, inserting the plugs into vials with the skin, which must be unblemished, uppermost, spraying uniformly, pushing the core home in the tube after drying, sealing the edge of the apple with wax and lead resinate and waxing the raw end. The tube is now ready to receive the codling-moth egg after which it is sealed. The tubes are set aside and examined later for the number of entrances, mortalities and stings, which can then be correlated with the concentration of poison used.

(c) *Fumigation experiments*

Bovingdon (1934) has reviewed the difficulties met with in this type of work. They are (a) the loss of gas from the fumigation chamber due to the introduction and removal of the insects, (b) the provision of means for ensuring uniform concentrations of the poisonous gas in the fumigation chamber by thorough mixing, (c) the determination of the gas concentration during an experiment, (d) the preparation of a gas mixture of a predetermined composition. He reviews the literature of the subject, gives brief descriptive summaries of the apparatus used by other workers in this field, and an account of a new type of apparatus for fumigating insects and the experimental procedure necessary for its use. The apparatus, which is of a somewhat complicated type, is housed in a constant temperature cabinet. By its use Bovingdon has in a very large measure overcome the difficulties mentioned above. The technique employed assures thorough mixing and circulation of the gas mixture and also secures that the test insects are subjected to the full concentration of the fumigant gas from the start of the experiment. The gas concentration in the fumigation flask is practically unaltered by the processes of introducing and removing the insects. If any criticism were to be offered on this ingenious apparatus it would concern its somewhat complicated nature and the rather involved technique requisite for its correct use.

Gough (1938 a) describes a modified form of the same apparatus in which Bovingdon and he have introduced a number of simplifications. The rocking of the apparatus provides for both circulation and mixing of the gas mixture. Gough also employed in his work a technique of a very simple type for circulating a fumigant-air mixture of known composition over insects, which to a great extent overcomes many of the difficulties met with in this work. The cleaning of the apparatus is easier, there are fewer stop-cocks the greasing of which may absorb some of the fumigant, the rubber-tubing exposed to the gas is reduced to a minimum and the exposure of the insects to mercury, which Gough (1938 b) has shown to have a toxic effect, may be largely avoided. The apparatus as so far developed does not permit, as in Bovingdon's

original type, of the insects being subjected to a full concentration of fumigant at the commencement of the experiment, and there may be a slight loss of fumigant on the introduction of the insects into the fumigation chamber. These difficulties do not seem insuperable, and owing to the large volume of the gas reservoir the proportional losses of fumigant due to the presence of the insects is minimized and accurate dosing is apparently rendered more easy.

Peters & Ganter (1935) and Peters (1936) describe a relatively simple apparatus, in which a series of fumigation chambers are arranged in parallel round a common gas distributor in a thermostat, humidity being kept constant. The amount of rubber-tubing and the number of corks exposed to the action of the gas would appear to be a drawback to its use. When the concentration-time relationships were examined, Haber's formula $c \times t = w$, which in effect states that toxic action is a product of concentration and time, was found approximately to fit the data obtained, a result in agreement with those of Mayer (1934) using ethylene oxide. A divergence from this equation arises apparently when low concentrations are used and at short exposures to high doses.

In general, for cases where exposures are given for definite times, after which the insect is taken from the apparatus and examined after a period of time in order to ascertain the effect, the formula $c^n t = w$ has been found to fit these types of data more closely. n usually varies between 1 and 2 and Haber's formula would appear to be a special case.

Busvine (1938) has reviewed and experimentally examined the factors affecting the action of fumigants and surveys the precautions to be taken in the laboratory assessment of their toxicity. He shows that different species of insects have a different order of resistance to different fumigants, that without adequate mixing and circulation of the gas mixture, considerable errors in determining the concentration of the fumigant may occur, and that despite great precautions in subjecting the insects rapidly to the action of the fumigant, there is a time lag before full concentration is attained, which, on the assumption of concentration × time being constant, he was able to calculate as a complete hiatus of 1·6 min. for his experimental conditions. The effect of temperature was a complicated one; physical properties such as vapour pressure, diffusion and sorption of the fumigant may play an important part. Toxicity is at the same time closely bound up with the physiological processes of the insect; rise in temperature as it increases metabolism can be correlated with increased susceptibility. The bearing of other variable factors on physiological condition and insect resistance are tabulated, and it is shown that, in general, factors increasing the rate of metabolic processes (as judged by respiration) increase susceptibility. Starvation, however, which lowers respiration rate, appears to decrease susceptibility to ethylene oxide, but to increase it to hydrogen cyanide.

In addition to dose-mortality relationships which are examined by the method of probits, Busvine analyses time-concentration data and finds that the logarithms of the times taken to kill 50 and 99 % of four different insects bears a linear relationship to the logarithms of the concentrations used. He claims that the formula $c^n t = w$ (where c = concentration in mg./l. N.T.P., t = time, and n represents the slope of the log c–log t regression line) gives a satisfactory representation of concentration-time curves for the four insects used as test subjects, a practical implication being that two exposure times [1] need only be investigated for its solution. He suggests the use of this formula for

[1] In the present state of our knowledge, it would appear advisable to have more than two times in order to verify that Busvine's formula fits the data.

expressing resistance of insects to a particular fumigant, the criteria requisite being concentrations which in a 5 hr. exposure secure 50 and 99% kill, and the value of *n*. The valid use of this formula would appear to necessitate the determination of probit-log concentration regression lines, since kills of 99% cannot be determined experimentally with any accuracy and are generally obtained by extrapolation. These lines should be straight and parallel over the range of exposure times. From Busvine's data one would draw the conclusion that his probit-log concentration regression lines do depart from parallelism. The net effect of this lack of parallelism on the determination of *n* for 50% kill does not appear to have been considerable, but the suggestion of applying the same constant to the 99% mortality values seems a highly doubtful one. Indeed, Bliss & Broadbent (1935) found a similar formula to fit their data for hydrocyanic gas as a fumigant for *Drosophila*, but noted that it changed from $c^{1.9}t = 10.2$ for a 50% mortality to $c^{1.3}t = 26.3$ for 97.5%, and they emphasize the importance of the level of mortality used in determining formulae of this type which have been somewhat generally used in toxicological work. It is perhaps advisable to remember that these curves, although they may be convenient for purposes of comparing relative rates of action, e.g. of drugs, appear to have provided singularly little information regarding the fundamental nature of their action (Clark, 1937).

Strand (1930), Lindgren & Shepard (1932), Shepard & Lindgren (1934) and Shepard *et al.* (1937) used a much simpler form of apparatus than those outlined above. They examined the mode of comparative assessment of insect fumigants. Their dosage-mortality curves are of the same sigmoid type as those obtained by workers engaged in the examination of the effect of sprays. The 50% mortality point or the median lethal dose is regarded as preferable for comparison, since it is more reproducible, but Shepard & Lindgren (1934) point out that, since the respective curves are not always parallel, a comparison of values near the 100% mortality, although only approximate, may be of greater practical value. Shepard (1934) suggests the use of the formula

$$x = K + k \cdot \log \frac{y}{100 - y}$$

for obtaining an estimate of the 99% lethal dose, where *x* and *y* are the values for dosage and mortality respectively, *K* the concentration required to produce 50% mortality and *k* the difference in the concentrations giving 90 and 50% mortalities.

Shepard *et al.* (1937) emphasize that a serious source of variation in results lies in the difficulty of distinguishing a sharp end-point, and that the latter should be so chosen that the estimated mortality will represent the eventual result for each species of insect as nearly as possible. They stress the importance of allowing for the recovery of resistant species as well as for tardy mortality induced by slow-acting chemicals.

Two methods for determining the toxicity of nicotine in the vapour phase are described by C. H. Richardson & Haas (1932) and by H. H. Richardson & Busbey (1937).

Gough's work (1938 *a*, *b*) indicates that care is not only needed in rearing test insects in a constant way, but that there is a marked effect on resistance to hydrocyanic acid gas of the time elapsing between the removal of certain insects from their food supply (in his case adults of *Tribolium confusum* in flour) and their fumigation. Those fumigated shortly after separation were less resistant than those separated for longer periods, males being less affected than females by early removal from flour. He also noted the emanation of a toxic substance from *Tribolium* adults, a result which

affected the type of container in which these insects could be placed for the purpose of fumigation. He observed cumulative losses of hydrocyanic acid gas in apparatus used previously for fumigating the adults, and the substance causing this loss could not be removed by evacuation. In addition, this worker's observation of the toxicity of mercury vapour to both the eggs of the bed bug and the confused flour beetle would indicate that exposure to this metal should be reduced to a minimum or, as Bovingdon suggests, gold leaf should be employed in the circuit to provide for its elimination from the fumigation chamber.

A CONSIDERATION OF SOME OF THE METHODS OF ASSESSING RESULTS

The toxic action of an insecticide may be quantitatively judged in three ways: (a) by the effect produced by different concentrations in a given time, (b) by the effect produced at different intervals of time, the concentrations being kept constant, (c) by the effects produced at different intervals of time by different concentrations. When plotted (a) gives rise to what are known as dosage-mortality curves, (b) to time-mortality curves, and (c) to time-concentration curves.

The curves produced are rarely linear and our knowledge of their nature and their interpretation has been greatly increased in recent years by the work of Osterhout (1922), Fisher (1924), Henderson-Smith (1921, 1923), Trevan (1927), Clark (1933, 1937), O'Kane et al. (1930, 1934), Hemmingsen (1933), Gaddum (1933), Bateman (1933), Bliss (1935 a, b, 1937) and others, for a full appreciation of which the original papers should be seen.

(a) Dosage-mortality curves

In general, it may be stated that the investigator of the insecticidal potency is mainly concerned with the ultimate death or recovery of the organism after the administration of the poison. Frequently, however, it is not possible to divide treated insects into two sharp divisions, those killed and those not affected. There are often gradations of toxic effect, the apparently dead, the moribund, those badly affected, slightly affected and those not affected, with graduations within each of these categories. It is necessary, therefore, to keep the treated insects under observation for a sufficient length of time in order to ascertain with some degree of certainty the ultimate effect, and, to have experience of the course narcosis may take. For example, in the case of pyrethrum an insect may recover from deep narcosis, whereas, after treatment with the rotenone-containing insecticides an insect, showing only partial paralysis, will gradually sink into a moribund condition. It is advisable for critical work, therefore, to fix upon certain symptoms as an aid to classification, and either to assess toxicity on the numbers apparently dead, or to group together the seriously affected insects, or to award marks for the different categories into which the treated insects group themselves. All these methods have been used with success. In the system of marking, so far, only arbitrary marks have been used (by Fryer et al. 1923 and by Worsley, 1934), but since it might well depend for its validity on the physiological action of the particular poison, a method of awarding marks based upon a statistical analysis of the data for each poison would appear to be called for.

The practical man wishes to know that concentration which will kill 100% of his organisms. Unfortunately, this is unattainable. When the concentrations used are plotted against the percentage kill or the percentage effect, as measured by some particular physiological action after a sufficient time has elapsed for a complete

response, the resulting curve is usually sigmoid or S-shaped, that is, it tails off at its upper and lower ends, where it approaches zero and 100% mortality, the change in percentage kill per unit of dose or concentration being greatest near the 50% kill. Fisher (1924) and Trevan (1927) have pointed out that for several different compounds the best point on these curves for purposes of comparison is at the concentration or dose securing 50% kill, which Trevan has named the *median lethal dose* or L.D. 50.

Although there has been discussion on the interpretation of these curves (see Clark, 1933), they are considered as being an expression of the variation in suscepti-bility between individual organisms drawn at random from the population. Were one able to express quantitatively the distribution of this susceptibility among a number of organisms the curve expected would be a normal frequency distribution (bell-shaped)—the S-shaped curve being its integrated or cumulative form. It has, however, been found that the susceptibilities are symmetrically distributed when the scale of dosages is logarithmically rather than arithmetically expressed.

It was independently observed by Bliss (1934), O'Kane *et al.* (1934), Gaddum (1933) and Hemmingsen (1933) that if the percentage of insects killed for several concentrations of a poison were equated to theoretical dosages, inferred from the cumulative normal curve, and expressed in units of standard deviations, and these units were plotted against the logarithms of the concentrations, a straight line resulted. These units have been termed "Normal Equivalent Deviations (N.E.D.)" by Gaddum, and "Probits" by Bliss. In Gaddum's terminology they are positive or negative, depending on whether the percentages are above or below 50%, which is 0. For ease of analysis, Bliss adds 5 units to each N.E.D. and thus gives them all positive values. Bliss (1935 *a*, *b*) has given much time to a consideration of this transformation, has published tables of probits, and of weights to be given to their values, since they are not uniform in this respect, the weight to be ascribed being a maximum at probit 5 (= 50% kill) and falling off towards the values for 0 and 100% kills. Bliss (1935 *a*, *b*) in his papers gives detailed examples of the use and the working-up of data by this method. The means of determining relative potencies of insecticides and drugs by its aid are given by Bliss (1935 *b*), by Irwin (1937) and, of certain insecticides, by Cochran (1938). For the essential tables and further refinements developed, *Statistical Tables for Biological, Agricultural and Medical Research*, by Fisher & Yates (1938), should be seen.

This linear transformation has greatly facilitated the study of insecticidal action. It has made possible the fuller use of the data accumulated for the purpose of com-paring toxicities, for, instead of having to rely upon one point on the curve, nearly all the points can be pooled and brought into use for purposes of assessment. It enables one to give an approximation to the concentration giving 100% effect and, by means of the χ^2 test, to determine the homogeneity of the data and goodness of fit of the regression line and to eliminate doubtful determinations. It is also possible to calculate the error in position and slope of the regression line. This technique, in short, gives an expression of the significance of the results.

(b) Time-mortality curves

Time-mortality data have been defined by Bliss (1937) as records of reaction time, which can be reduced to a form showing the number of organisms reacting to a toxicological stimulus in each of several successive periods of time. It was shown by

Henderson-Smith (1921, 1923) that such curves (usually S-shaped in their cumulative form) are, as in the case of dosage-mortality curves, explicable on the assumption that individual organisms differ in resistance and that the distribution in resistance grades approaches the normal frequency curve. These curves have considerable usefulness in giving information on toxicological problems (e.g. in fumigation), although it is generally agreed that for most practical purposes the dosage-mortality curve is more informative. If, for example, one were to assess the comparative toxicities of pyrethrum and derris by determining the time at which a number of insects were paralysed, one might arrive at a wholly erroneous estimate of their relative values as insecticides, since one is rapid in action with a tendency towards recovery, and the other slow in action with a tendency towards deepening paralysis. Bliss, who has examined statistically time-reaction curves, considers that they are often of indeterminate value unless they can be transformed to straight lines by conversion of percentages to probits and the observed time to logarithms or rates.

(c) Time-concentration curves

These have been often used in toxicological work. As frequently expressed, they result in the replication on the same diagram of curves giving the relationship between time and percentage of organisms killed or surviving for a number of concentrations, and are commonly used to determine the rate of toxic action. This can readily be done by taking the reciprocals of the time required to produce the same effect. If the toxic effects for the various concentrations are determined after a number of different periods and complete curves are drawn, no difficulty is likely to be met with in determining the rates of action. If this is not possible then a careful choice of procedure is needed. Henderson-Smith (1923) and Osterhout (1922) have pointed out that the rate of action can be regarded from two points of view, (a) the toxic effect produced in a given time, (b) the time taken to produce a given toxic effect. In biological reactions the rate rarely proceeds at uniform speed and as determined by the first of these alternatives may lead to serious errors of assessment. This can be clearly seen if two unimolecular reactions, one proceeding at twice the rate of the other, are plotted. The same generalization can be drawn with respect to the determination of temperature coefficients as they affect rate of toxic action. Henderson-Smith's investigation (1921) on the toxicity of phenol to *Botrytis* spores shows also that the shape of the curves in which percentage kill is plotted against time may alter with concentration, and thus the ratios of the time taken to kill varying proportions, e.g. 25, 50, 75%, for several concentrations of poison lead to different results. Since, however, the greatest uniformity in the rate of response of a population of organisms is found in the region of the 50% effect, this point is the best one for assessing rate of toxic action.

Campbell (1930) considered that the most nearly complete picture of the acute toxicity of a compound would be obtained by plotting the reciprocals of the recovery periods and of the survival periods against doses covering as wide a range as possible. This method would give a graph resembling the cross-section of a valley, one side representing the effect of sublethal doses, the other representing the effect of lethal doses and the bottom of the valley representing the region of the median lethal dose. For comparing insecticides, however, he plotted the speed or rate of toxic action (i.e. the reciprocal of the time taken to paralyse or to kill) against the dosage. Such curves, often of a sigmoid character, were not regarded as owing their character to variations

in individual susceptibility (1926 a). By measuring the area below the curves given by the respective compounds he assessed relative toxicity.

Bliss (1936) examined in detail some of Campbell's data (1926 a, b) showing the change of rate of toxic action with oral dosage of arsenic when silkworms were used as a test subject. Several important points were brought to light. In oral experiments, the dosage is most commonly represented as the weight of poison administered per body weight; this practice was examined by Bliss. His analysis showed that the rate of toxic action seemed to be a linear function of the log of the dose per larva and the log of the body weight. The power of the body weight W^h, however, which eliminated the effect of body size upon the rate of toxic action, depended for its magnitude upon whether the speed of toxic action or its log were used in computation. In the former case h increased from 1·67 to 1·96 as one passed from the 2nd to the 5th instars, but when the log rate was used h was the same for all instars and averaged 1·5. Thus for all instars of the silkworm, when the log rate of toxic action was plotted against the adjusted dosage, $\dfrac{\text{mg. arsenic}}{(\text{g. body weight})^{1 \cdot 5}}$, expressed in logarithms, a straight line resulted. If the separate instars were taken and the rate of toxic action was plotted against the log dose, $\dfrac{\text{mg. arsenic}}{(\text{g. body weight})^{1 \cdot 67 - 1 \cdot 96}}$, straight lines were also obtained for each instar. If it could be assumed in these cases that poisoning followed the same course at both low and high dosages, and therefore, that the individual rates of toxic action would be symmetrically distributed about the curves throughout their course, theoretically it should follow, that the point at which each straight line intersects the base line should represent the median lethal dose for the particular instar. Such a deduction, however, requires experimental confirmation.

FIELD EXPERIMENTS

Until recent years the usual method of assessing results of insecticidal trials in the field was by visual inspection. Now, with the increasing use of appropriately designed experiments, statistical analysis is yielding quantitative data which give a measure of the significance of the results obtained. The aim of the experiment may be to ascertain the effect upon crop yield or an estimate of the degree of control of the pest, involving counts of the latter, or a combination of the two methods. The experiment may take the form of the randomized block or of the Latin square, with the subsequent use of the analysis of variance technique for the determination of the significance of the results. Bartlett (1936) has pointed out, however, that if direct counts of pests are to be made, a careful examination of the suitability of the design may be needed. The statistical analysis may also require special treatment.[1] It is outside the scope of this paper to deal with this complex subject, but it should be indicated that before field experiments are carried out on the control of insect pests by insecticides, the experiment should be soundly designed from a statistical point of view in order to yield a maximum of information. Two recent examples of the use of the Latin square technique are given by Gaines (1937 a, b). An experiment, arranged in randomized blocks, in which derris and nicotine were tested for the control of raspberry beetle (*Byturus tomentosus*), was carried out by Steer (1933) and yielded results of considerable economic importance.

[1] See also Cochran, W. G., "Some difficulties in statistical analysis", *Emp. J. exp. Agric.* (1938), **6**, 157–75.

REFERENCES

BADERTSCHER, A. E. (1936). Insecticide tests compared. *Soap*, **12**, no. 9, 96.

BARTLETT, M. S. (1936). Some notes on insecticide tests in the laboratory and in the field. *Suppl. J.R. statist. Soc.* **3**, 185.

BATEMAN, E. (1933). The effect of concentration on the toxicity of chemicals to living organisms. *Tech. Bull. U.S. Dep. Agric.* no. 346.

BLISS, C. I. (1934). The method of probits. *Science*, **79**, 38, 409.

—— (1935 a). The calculation of the dosage-mortality curve. *Ann. appl. Biol.* **22**, 134.

—— (1935 b). The comparison of dosage-mortality data. *Ann. appl. Biol.* **22**, 307.

—— (1936). The size factor in the action of arsenic upon silkworm larvae. *J. exp. Biol.* **13**, 95.

—— (1937). The calculation of the time-mortality curve. *Ann. appl. Biol.* **24**, 815.

BLISS, C. I. & BROADBENT, B. M. (1935). A comparison of criteria of susceptibility in the response of *Drosophila* to hydrocyanic acid gas. I. *J. econ. Ent.* **28**, 989.

BOVINGDON, H. H. S. (1934). An improved laboratory apparatus for fumigation experiments. *Ann. appl. Biol.* **21**, 704.

BULGER, J. W. (1932). Additions to our knowledge of the toxicity of stomach-poisons to insects. *J. econ. Ent.* **25**, 261.

—— (1935). A photoelectric method for measuring small leaf areas. *J. econ. Ent.* **28**, 76.

(1937). Feeding predetermined doses of poison to silkworm. *J. econ. Ent.* **30**, 689.

BUSVINE, J. R. (1938). The toxicity of ethylene oxide to *Calandra oryzae, Calandra granaria, Tribolium castaneum*, and *Cimex lectularius. Ann. appl. Biol.* **25**, 605.

CAMPBELL, F. L. (1926 a). The speed of toxic action of arsenic in the silkworm. *J. gen. Physiol.* **9**, 433.

—— (1926 b). Relative susceptibility to arsenic in successive instars of the silkworm. *J. gen. Physiol.* **9**, 727.

—— (1926 c). The practicability of quantitative toxicological investigations on mandibulate insects. *J. agric. Res.* **32**, 359.

—— (1930). A comparison of four methods for estimating the relative toxicity of stomach-poison insecticides. *J. econ. Ent.* **23**, 357.

—— (1938). Co-operative tests of housefly sprays, 1935–36. *U.S. Dept. Agric. Bur. Ent. and Plant Quar.*, Mimeographed Circ. E. 436.

CAMPBELL, F. L. & FILMER, R. S. (1929). A quantitative method of estimating the relative toxicity of stomach-poison insecticides. *Trans. IV Internat. Cong. Ent. for 1928*, 523.

CAMPBELL, F. L. & SULLIVAN, W. N. (1938). A metal turn-table method for comparative tests of liquid spray contact insecticides. *Soap*, **14**, no. 6, 119.

CLARK, A. J. (1933). *The Mode of Action of Drugs on Cells*. London: Edward Arnold and Co.

—— (1937). *General Pharmacology*, p. 132. Berlin: Julius Springer.

COCHRAN, W. G. (1938). Appendix to a paper by TATTERSFIELD, F. & MARTIN, J. T. The problem of the evaluation of rotenone-containing plants. IV. *Ann. appl. Biol.* **25**, 411.

CRAUFURD-BENSON, H. J. (1938). An improved method for testing liquid contact insecticides in the laboratory. *Bull. ent. Res.* **29**, 41.

FISHER, R. A. (1924). Note in TATTERSFIELD, F. & MORRIS, H. M. An apparatus for testing the toxic values of contact insecticides under controlled conditions. *Bull. ent. Res.* **14**, 223.

FISHER, R. A. & YATES, F. (1938). *Statistical Tables for Biological, Agricultural and Medical Research.* Edinburgh: Oliver and Boyd.

FLEMING, W. E. (1934). Development of a standard cage method for testing the effectiveness of stomach-poison insecticides on Japanese beetle. *J. agric. Res.* **48**, 115.

FLEMING, W. E. & BAKER, F. E. (1934). Testing contact insecticides on Japanese beetle. *J. agric. Res.* **49**, 29.

FRYER, C. F., STENTON, R., TATTERSFIELD, F. & ROACH, W. A. (1923). A quantitative study of the insecticidal properties of *Derris elliptica. Ann. appl. Biol.* **10**, 18.

GADDUM, J. H. (1933). Methods of biological assay depending on a quantal response. Reports on Biological Standards. III. *Spec. Rep. Ser. med. Res. Coun., Lond.,* no. 183.

GAINES, J. C. (1937 *a*). Cotton flea hopper control tests. *J. econ. Ent.* **30**, 119–25.

—— (1937 *b*). Tests of insecticides for cotton-boll weevil and bollworm control. *J. econ. Ent.* **30**, 785.

GIMINGHAM, C. T., MASSEE, A. M. & TATTERSFIELD, F. (1926). A quantitative examination of the toxicity of 3:5 dinitro-*o*-cresol and other compounds to insect eggs. *Ann. appl. Biol.* **13**, 446.

GIMINGHAM, C. T. & TATTERSFIELD, F. (1928). Laboratory experiments with non-arsenical insecticides for biting-insects. *Ann. appl. Biol.* **15**, 649.

GOETZE, G. (1932). Zwei neue Methoden zum quantitiven Studium der Giftigkeit von Insektiziden. *Anz. Schädlingsk.* **8**, 54. [*R.A.E.* (1932), A, **20**, 442.]

GÖRNITZ, K. (1933). Methoden zur Prüfung von Pflanzenschutzmitteln. IV. *Mitt. biol. Reichsanst. Berl.* no. 46, 1. [*R.A.E.* (1933), A, **21**, 385.]

GOUGH, H. C. (1938 *a*). Physiological and environmental factors affecting the resistance of insects to toxic substances, with a special reference to the resistance of the confused flour beetle, *Tribolium confusum* Duv. to hydrogen cyanide. Ph.D. Thesis. The University of London (shortly to be published).

—— (1938 *b*). Toxicity of mercury vapour to insects. *Nature, Lond.,* **141**, 922.

HARTZELL, A. & WILCOXON, F. (1932). Chemical and toxicological studies of pyrethrum. *Contr. Boyce Thompson Inst.* **4**, no. 1, 107.

HEMMINGSEN, A. M. (1933). The accuracy of insulin assay on white mice. *Quart. J. Pharm.* **6**, 39.

HENDERSON-SMITH, J. (1921). Killing of *Botrytis* spores by phenol. *Ann. appl. Biol.* **8**, 27.

—— (1923). The killing of *Botrytis cinerea* by heat. *Ann. appl. Biol.* **10**, 335.

IRWIN, J. O. (1937). Statistical method applied to biological assays. *Suppl. J.R. statist. Soc.* **4**, 1–60.

JANISCH, R. (1926). Eine neue Methode zur vergleichenden Beurteilung der Wirksamkeit von Insektenfrassgiften. *NachrBl. dtsch. PflSch-Dienst.* **6**, 18. [*R.A.E.* (1926), A, **14**, 192.]

JONES, H. A., CAMPBELL, F. L. & SULLIVAN, W. N. (1935). Cracca, a source of insecticides. *Soap,* **11**, no. 9, 101.

KEARNS, H. G. & MARTIN, H. (1936). Investigations on egg-killing washes. *Long Ashton Res. Sta. Ann. Rep. for* 1935, p. 49.

LINDGREN, D. L. & SHEPARD, H. H. (1932). The influence of humidity on the effectiveness of certain fumigants against eggs and adults of *Tribolium confusum* Duv. *J. econ. Ent.* **25**, 248.

MAYER, K. (1934). Die letale Dosis Äthylenoxid bei *Calandra granaria, Tribolium confusum,* und *Cimex lectularius. Arb. physiol. angew. Ent.* **1**, 257. [*R.A.E.* (1935), A, **23**, 121.]

MILLER, A. C. & SIMANTON, W. A. (1938). Biological factors in Peet-Grady results. *Soap,* **14**, no. 5, 103.

MURRAY, C. A. (1937). A statistical analysis of fly mortality. *Soap,* **13**, no. 8, 88.

MURRAY, C. A. (1938). Dosage-mortality in the Peet-Grady method. *Soap*, **14**, no. 2, 99.

NELSON, F. C., BUC, H. E., SANKOWSKY, N. A. & JERNAKOFF, M. (1934). Evaluating liquid insecticides. *Soap*, **10**, no. 10, 85.

NEWCOMER, E. J. (1926). Laboratory experiments with arsenicals in the control of codling moth. *J. agric. Res.* **33**, 317.

O'KANE, W. C., WALKER, G. L., GUY, H. G. & SMITH, O. J. (1933). Studies of contact insecticides. VI. *Tech. Bull. N.H. agric. Exp. Sta.* no. 54.

O'KANE, W. C., WESTGATE, W. A. & GLOVER, L. C. (1934). Studies of contact insecticides. VII. *Tech. Bull. N.H. agric. Exp. Sta.* no. 58.

O'KANE, W. C., WESTGATE, W. A., GLOVER, L. C. & LOWRY, P. R. (1930). Studies of contact insecticides. I. *Tech. Bull. N.H. agric. Exp. Sta.* no. 39.

OSTERHOUT, W. J. V. (1922). *Injury, Recovery and Death in Relation to Conductivity and Permeability*, p. 69. J. B. Lippincott Co. (Monographs on Experimental Biology).

PEET, C. H. (1932). The Peet-Grady method revised. *Soap*, **8**, no. 4, 98.

PEET, C. H. & GRADY, A. G. (1928). Studies in insecticidal activity. *J. econ. Ent.* **21**, 612.

PETERS, G. (1936). *Chemie und Toxikologie der Schädlingsbekämpfung. Samml. chem. u. chem.-techn. Vorträge*, no. 31, Stuttgart.

PETERS, G. & GANTER, W. (1935). Zur Frage Abtötung des Kornkäfers mit Blausäure. *Z. angew. Ent.* **21**, 547. [*R.A.E.* (1935), A, **23**, 258.]

PRICE, W. A. (1920). Bees and their relation to arsenic sprays at blossoming time. *Bull. Ind. agric. Exp. Sta.* no. 247.

RICHARDSON, C. H. & HAAS, L. E. (1932). The relative toxicity of pyridine and nicotine in the gaseous condition to *Tribolium confusum*. *Iowa St. Coll. J. Sci.* **6**, 287.

RICHARDSON, H. H. (1931). Insecticidal method for the estimation of kerosene extracts of pyrethrum. *J. econ. Ent.* **24**, 97.

—— (1932). An efficient medium for rearing houseflies throughout the year. *Science*, **76**, 350.

RICHARDSON, H. H. & BUSBEY, R. L. (1937). Laboratory apparatus for fumigation with low concentrations of nicotine. *J. econ. Ent.* **30**, 576.

SHEPARD, H. H. (1934). Relative toxicity at high percentages of insect mortality. *Nature, Lond.*, **134**, 323.

SHEPARD, H. H. & LINDGREN, D. L. (1934). The relative efficiency of some fumigants against rice weevil and confused flour beetle. *J. econ. Ent.* **27**, 842.

SHEPARD, H. H., LINDGREN, D. L. & THOMAS, E. L. (1937). The relative toxicity of insect fumigants. *Tech. Bull. Minn. agric. Exp. Sta.* no. 120.

SHEPARD, H. H. & RICHARDSON, C. H. (1931). A method for determining the relative toxicity of contact insecticides. *J. econ. Ent.* **24**, 905.

SIEGLER, E. H. & MUNGER, F. (1933). A field and laboratory technique for toxicological studies of the codling-moth. *J. econ. Ent.* **26**, 438.

SIMANTON, W. A. (1937). Evaluating liquid insecticides. Comments on the 1937 official method and use of the official control insecticide in grading liquid housefly sprays. *Soap*, **13**, no. 10, 103.

SIMANTON, W. A. & MILLER, A. C. (1937). Housefly age as a factor in susceptibility to pyrethrum spray. *J. econ. Ent.* **30**, 917.

—— (1938). Greater speed and accuracy with modified Peet-Grady method. *Soap*, **14**, no. 5, 115.

STEER, W. (1933). Studies on *Byturus tomentosus*. Fabr. III. *J. Pomol.* **11**, 19.

—— (1938). Laboratory methods for the biological testing of insecticides. I. *J. Pomol.* **15**, 338.

STELLWAAG, F. (1931). Giftigkeit und Giftwert der Insecticide. VI. *Z. angew. Ent.* **18**, 113–32. [*R.A.E.* (1931), A, **19**, 698.]

STRAND, A. L. (1930). Measuring the toxicity of insect fumigants. *Industr. Engng Chem.* (Anal. Ed.), **2**, 4.

TATTERSFIELD, F. (1934). An apparatus for testing contact insecticides. *Ann. appl. Biol.* **21**, 691.

TATTERSFIELD, F. & MORRIS, H. M. (1924). An apparatus for testing the toxic values of contact insecticides under controlled conditions. *Bull. ent. Res.* **14**, 223.

THALENHORST, W. (1937). Versuche über die Wirkung von Kontaktstäubemitteln auf *Pieris brassicae. Z. angew. Ent.* **23**, 615. [*R.A.E.* (1937), A, **25**, 501.]

TRAPPMANN, W. & NITSCHE, G. (1934). Eine einfache Dosierungsvorrichtung für Spritzmittel bei Laboratoriums-Versuchen. *NachrBl. dtsch. PflSchDienst.* **14**, 51. [*R.A.E.* (1934), A, **22**, 385.]

TREVAN, J. W. (1927). The error of determination of toxicity. *Proc. roy. Soc.* B, **101**, 483.

TUMA, V. (1938). A study of the relationship between the ages of cockroaches and their resistance to liquid insecticides. *Soap,* **14**, no. 6, 109.

WORSLEY, R. R. (1934). The insecticidal properties of some East African plants. I. *Ann. appl. Biol.* **21**, 649.

ZERMUEHLEN, A. E. & ALLEN, T. C. (1936). Modified procedure in testing petroleum base insecticides by the settling mist method. *Soap,* **12**, no. 6, 105.

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

ANNUAL GENERAL MEETING of the Association of Applied Biologists held in the Imperial College of Science and Technology, London, on Friday, 10 February 1939. The morning session began at 11.45 a.m. in the Botany Lecture Theatre, and the afternoon session at 2.30 p.m. in the Metallurgy Lecture Theatre. The Chair was taken by the President, Mr C. T. GIMINGHAM.

Discussion on Sugar Beet Problems

The following papers were read:

I. Acidity and manganese deficiency problems in connexion with sugar beet growing. By W. MORLEY DAVIES, M.A., B.Sc.

II. The effect of boron on the growth and quality of sugar beet. By A. W. GREENHILL, Ph.D.. M.Sc., F.I.C., A.R.C.S.

III. Sugar beet pests. By F. R. PETHERBRIDGE, M.A.

IV. Pests of the sugar beet crop in the Midlands. By A. ROEBUCK, N.D.A.

V. Sugar beet diseases. By H. H. STIRRUP, M.Sc.

I. ACIDITY AND MANGANESE DEFICIENCY PROBLEMS IN CONNEXION WITH SUGAR BEET GROWING

BY W. MORLEY DAVIES, M.A., B.Sc.

Advisory Chemist and Head of Chemical Department, Harper Adams Agricultural College, Newport, Shropshire

Acidity

SUGAR BEET may be regarded as one of the acid sensitive crops and falling into the same class as barley, clover and mangolds in this respect. In the earlier days of beet growing in this country acidity doubtless accounted for at least 75% of the failures of the crop. Gradually, however, farmers have come to realize that the presence of non-acid conditions are essential and nowadays it is the exception to find many failures from this cause. Being a deep-rooting plant and carrying on some of its feeding in the subsoil, adequate preparation in the way of liming an acid soil should be made some time before the crop is grown. Many growers think that they have done sufficient if they give a dressing of lime after ploughing—lightly cultivating it into the surface—and numerous cases of this practice have come to the writer's notice. Under these conditions short stubby plants are obtained with innumerable side rootlets giving a

hairy appearance. It is essential that the lime should be applied two or three years previously, if the soil is at all acid, so that it will have an opportunity to penetrate to the subsoil.

Under acid soil conditions the seedling usually grows fairly satisfactorily as far as the fourth leaf stage. Thereafter, the plant remains stunted throughout the season or dies. The small plants have a rather characteristic dark green appearance and often develop a red colour at the base of the petioles. Under still more acid conditions the seedling may not grow beyond the cotyledon stage, shrivelling in dry weather. Under less severe, but still somewhat acid conditions, the crop may grow fairly normally without, however, yielding satisfactorily. Acidity seems to have no very marked influence on the sugar percentage but reduces the weight of sugar per acre in proportion to the diminution in yield.

Soil conditions

Under excessively acid conditions the crop will fail more or less completely. This condition, however, is not often seen under farming conditions, since, in the field, acidity manifests itself by a patchy crop. The changes from favourable to unfavourable conditions are often sharply defined. The usual field symptoms are either patches where the beets are diminutive, or patches where the plant is "gappy". From the examination of numerous fields and by comparisons of good and poor growth in those fields the crop would appear to fail below a critical pH of about 5·3. It is interesting to note that failures at this figure have been recorded as far apart as the Midlands, the North of Scotland and Southern Ireland. Precision in stating any absolute value for such a figure is not claimed since, doubtless, it varies somewhat with different soil and climatic conditions.

The results of two experiments which have been carried out in the West Midlands can be cited to illustrate the effect of acid soil conditions on yields.

Liming experiment at Harper Adams College

In 1928 an experiment in the form of a Latin square and including four graded treatments with lime was laid down on a sandy soil. This has now been conducted over ten years without further additions of lime. In 1930 sugar beet was grown and the results for that year are given in Table I.

Table I

Dressing cwt. CaCO$_3$/acre	Yield of beet tons/acre	% sugar	pH	Exchangeable CaO
0	4·5	16·7	4·9	0·089
25	9·1	17·1	5·3	0·114
50	10·25	17·1	5·5	0·115
100	10·1	17·2	5·9	0·161

Lime requirement, Hutchinson & McLelland, 40 cwt./acre CaCO$_3$.

The results show that the effect of the smallest dressing of lime substantially and significantly increased the crop. Thereafter, no significant increases were produced by successive dressings. The results also show that the critical acidity value is about 5·3 and an exchangeable lime content of about 0·09% (calcium oxide). The latter figure would doubtless vary considerably on different soil types. There was no very marked relation between the sugar percentage and the base status of the soil.

Experiment using sugar beet factory waste lime

Another experiment which throws light on the effects of acidity and subsequent liming on the yield of beet and mangolds was carried out in the West Midlands. In this, sugar beet factory waste lime (sludge and dried) was tested against a standard ground limestone. The results are shown in Table II.

Table II

Yield of crops

| | Sugar beet (1935) | | Mangolds (1936) Roots tons/acre | Sugar beet (1937) | |
	Roots (washed) tons/acre	Sugar cwt./acre		Roots (washed) tons/acre	Sugar cwt./acre
Control	9·76	33·14	17·83	4·30	13·2
Half-ground limestone	11·28	38·12	20·21	7·04	22·5
Full-ground limestone	10·74	36·16	20·68	7·94	25·0
Half-dried sludge	11·31	38·14	20·52	7·47	23·7
Full-dried sludge	12·05	41·10	22·18	8·88	28·5
Half-wet sludge	11·02	37·54	20·07	6·67	21·1
Full-wet sludge	11·22	38·46	20·80	8·19	26·4
Total averages	11·03	37·52	20·33	7·21	22·9
Limed plots, averages	11·27	38·25	20·41	7·70	24·5

Soil reaction (pH)

	Initial, 1934	1935	1936	1937
Control	5·4	5·4	5·1	5·0
Half-ground limestone		5·8	5·5	5·2
Full-ground limestone		5·9	5·5	5·5
Half-dried sludge		5·8	5·5	5·3
Full-dried sludge		6·3	5·8	5·6
Half-wet sludge		5·8	5·6	5·5
Full-wet sludge		6·1	5·7	5·5

Liming with ground limestone, dried sludge and wet sludge produced marked increases in yield both at full-dressings and half-dressing rates. The increases resulting from liming were not so great as in the previously quoted experiment since the soil was initially less acid.

Manganese deficiency

Troubles associated with manganese lie on another part of the acidity scale and only occur, at any rate in this country, under conditions tending to alkalinity.

Appearance of the crop. Sugar beet and mangolds. Both crops seem sensitive to manganese deficiency and exhibit a condition referred to in beet as "Speckled Yellows". For a time, after singling, the plants appear to be quite normal, then, within a few days, a yellowing of the foliage becomes apparent. This happens when the plant is about 6 in. high and usually in the months of June or July. Closer examination of the foliage shows the presence of numerous yellowish blotches occurring in the interveinal tissue. By transmitted light these are particularly noticeable and stand out in comparison with the darker green of the veins. At a subsequent stage this blotched tissue dies and may or may not fall out of the leaf. It is possible that two conditions of these symptoms occur, (a) where the blotches just turn yellow and remain so, and (b) where they turn yellow, then brown, eventually dying and falling away from the

leaf. Many of the affected leaves, but not by any means all, assume a triangular shape due to the rolling of the edges towards the centre. Later in the season there is considerable recovery, the younger leaves being less noticeably yellow, and towards autumn the crop appears normal.

Soil conditions conducive to manganese deficiency. Two soil conditions seem necessary, in this country at any rate, for the production of the disease in manganese sensitive crops: (*a*) a reaction value which exceeds *p*H 6·5, and (*b*) the presence of considerable amounts of organic matter. These conditions must occur at one and the same time. Seldom do the symptoms appear in well-limed mineral soils of normal or low organic matter content, neither do they occur in soils of high organic content unless excessive liming or natural conditions give rise to a high reaction value. There should be no difficulty in recognizing soils in which the disease may appear, since they are usually black or dark grey in colour. Peat soils, and humus-sands occurring on comparatively recently reclaimed heath and moorland are the main types on which the disease occurs. Cases are known where certain fields are naturally subject to the disease. Among those cases of the occurrence of Speckled Yellows reported by Cranfield, one occurred in a field where a shallow fen soil overlaid a chalk gravel, and another where a peaty sand overlaid calcareous gravels of the Old Trent Valley. In those cases the top-soil reaction was naturally and permanently maintained above *p*H 7.

Various hypotheses have been put forward to account for the effect of alkalinity in rendering the manganese unavailable in soils rich in organic matter. Some investigators conclude that under alkaline conditions the more easily available or bivalent form passes to a less readily available condition of a higher oxide. The close association of manganese deficiency with soils rich in humus suggests that the manganese becomes tied up in some way with the organic complexes. Piper, working in Australia, found that the symptoms appeared on oats when the *p*H exceeded 6·7, especially on heavily limed black sands (sandy podsols).

The figures in Table III were obtained from three affected areas in an experimental field near Tamworth and refer to oats.

Table III. *Relation between soil conditions and Grey Speck in oats (July* 1936)

	Area A		Area B		Area C	
	*p*H	Exchangeable CaO %	*p*H	Exchangeable CaO %	*p*H	Exchangeable CaO %
Crop good	6·2	0·41	6·5	0·40	6·5	0·46
Crop somewhat affected	6·6	0·44	6·7	0·42	6·5	0·47
Crop severely affected	7·3	0·52	7·3	0·46	6·9	0·48

Manganese appears unique among the exchangeable bases in that it may pass from the active form into the unavailable higher oxide or vice versa, according to the balance of the oxidation-reduction equilibrium prevailing in the soil. This balance appears primarily to be determined by the soil reaction.

An interesting paper by Gerretsen of Holland provides evidence that Grey Speck of oats may not be due solely to manganese deficiency. Gerretsen believes that the disease is bacterial. Plants with abnormal metabolism due to lack of available manganese are stated to be unable to synthesize organic acids in sufficient quantity

to neutralize ammonia produced by the bacteria in the vicinity of the roots. Lack of carbohydrates in the leaves weakens them so that they bend in a characteristic manner. Vigorous plants growing under normal conditions are little affected by the bacteria. If this be the case then the symptoms associated with Grey Speck must be regarded as secondary and not primarily due to manganese deficiency.

Control of the disease

Measurements on the effect of soil treatment in the resulting crops have been made, more particularly on oats. The results of satisfactory forms of treatment are however equally applicable to sugar beet.

Under field conditions several methods of control are being investigated:

(1) Treatment with compounds containing manganese applied either to the soil as a top dressing or as a spray on the foliage, such as manganese sulphate, basic slag, etc.

(2) Introduction of acidifying conditions by treating the soil with sulphur, sulphate of ammonia, etc.

(3) Introduction of reducing conditions by incorporation of farmyard manure and chemical substances, hydroquinone, sodium sulphite, etc.

Treatment with compounds containing manganese

Of these compounds manganous sulphate seems to be the one most widely used. Experiments have also been conducted with other salts of manganese, notably the chloride. In addition, basic slag, as a source of manganese (9.3% manganous oxide), has been used with some success.

Results obtained by Morley Davies & Irons on oats, cut whilst still green, near Tamworth in Warwickshire with manganese sulphate and basic slag in 1934 and 1935 are shown in Table IV.

Table IV. *Effect of slag and manganous sulphate in the control of Grey Speck of oats*

	Yield of dry matter (cwt./acre)		Mn uptake (lb./acre)	
	1934*	1935†	1934	1935
Control	20·8	39·2	0·57	0·35
Slag (6 cwt./acre)	21·7	42·2	0·65	0·43
Slag (12 cwt./acre)	23·8	43·2	0·59	0·51
$MnSO_4$ (56 lb./acre)	27·1	47·2	0·94	0·73
$MnSO_4$ (112 lb./acre)	30·6	46·5	1·44	0·83
Significant differences are greater than	3·54	3·94	—	0·18

* Air-dried matter.   † Absolute dry matter.

Stewart investigated a case of manganese deficiency in oats at Holmeswood, Lancashire, where the soil pH was 6·9. Treatment with manganous sulphate and basic slag resulted in increased uptake of manganese with the heavier dressings (Table V).

The effects of manganese sulphate and slag as control materials in improving the crop are corroborated by more recent observations on plots laid down by Morley Davies, Bates & Tilley near Stourbridge. Here the effect of slag was more pronounced on crops less susceptible to the disease, such as barley, than on oats, but was in no case so effective as manganese sulphate. It was interesting to note that Marsh

Spot in peas (1938) was controlled at the same centre only by dressing with manganese sulphate, other forms of treatment proving ineffective.

Table V.   *The effect of dressings of manganous sulphate and slag on the uptake of manganese in oats*

Treatment	Mn_3O_4 % in dry matter of crop
1. Control	0·0021
2. Manganese sulphate 56 lb./acre	0·0020
Manganese sulphate 112 lb./acre	0·0041
3. Basic slag 5 cwt./acre	0·0018
Basic slag 10 cwt./acre	0·0034

Treatment with acidifying materials

Sulphur, incorporated with the soil, has been widely used as an agent for acidification. It reduces the soil reaction and consequently increases the supply of available manganese. Plots at Stourbridge were dressed with sulphur at the rate of 1 ton/acre and were rendered markedly more acid, the pH being reduced by the single dressing in 1937 from 6·8 to 5·7; this was maintained at pH 6·0 in 1938 without further dressing. The control of the disease in all sensitive crops was good and was doubtless due to an increase in the readily available manganese, viz. from 0·14 to 0·27 mg./100 g. soil.

Sulphate of ammonia was also tried in 1938 at the same centre, but, whether owing to the dry conditions of the season, or for some other cause, did not prove nearly so effective as sulphur.

Introduction of reducing conditions

Piper's work has shown that temporary waterlogging of plants grown in soil in pots had a marked effect on eliminating symptoms of the disease. This he attributes to the introduction of conditions which resulted in the reduction of the more highly oxidized and unavailable materials to a soluble and available state. In this country Godden & Grimmett found that oats grown in pots without drainage contained about six times as much manganese as those in pots provided with drainage, indicating that reducing conditions increased the solubility of the manganese. In practice this would be difficult to achieve except under conditions where irrigation could be controlled and, even then, might not be effective.

The effect of incorporating farmyard manure with the soil is marked by a reduction of the symptoms shown by the plant. While this effect is thought to be brought about by the introduction of reducing conditions, it is by no means an established fact and further investigation is necessary.

So far no satisfactory field control has been achieved by applying chemical reducing compounds as distinct from acidifying agents.

Data referring to the composition of the plant under normal and manganese deficient conditions are now being accumulated in this country. Table VI shows the effect of treatment on the manganese content of roots, laminae and petioles of mangolds.

Distribution of the disease

An examination of the voluminous literature dealing with manganese deficiency and its control suggests that the disease is widespread. The countries where it appears

Table VI. *Manganese content (p.p.m. of Mn) in dry matter*

	Mangolds		
	Roots	Laminae	Petioles
Control	18	20	16
Artificials	24	30	15
MnSO$_4$ + artificials	40	180	46
MnSO$_4$ alone	26	86	20

to be of importance occur both in the new and old worlds and include America, Australia, Japan, Holland, Scandinavia, Great Britain and many others. Just how important it is in our own country it is difficult to say, but there is no doubt that both peat, and mineral soils high in organic matter such as are found in reclaimed heath and moorland areas, are fairly extensive, particularly in certain localities. The area of fenland alone exceeds a million acres. The disease is associated with definite soil types, such as would be identified and mapped by the soil survey. This, among many other good reasons, points to the necessity for increasing the activity of soil surveys in this country and, until that is done, the actual area where manganese deficiency diseases may occur under natural or artificially induced conditions must remain a matter of speculation.

Practical implications: danger of over-liming

No soils, so far, have been discovered in this country in which manganese is absent. Certainly, some contain very low amounts (0·004%), but under suitable conditions these are adequate for the plants' needs. It has been shown that raising the reaction of a soil above a *p*H value of 6·5 is conducive to the occurrence of disease. This effect is not generally apparent in ordinary mineral soils but occurs in those with a high organic content. No definite statement as to the amount of organic matter which must be present can be made, since this figure will vary according to the amount of the mineral part of the soil. Black sands, on which the disease is present, may have an organic matter content as low as 6%. Peat soils, on the other hand, have an extremely variable organic matter content and may be as high as 90%. Certain natural conditions, such as the occurrence of an alkaline subsoil, lead to the appearance of the disease in crops and are largely outside the control of the farmer. The rate of application of lime, however, does come under the control of the farmer and it is essential that care be exercised on susceptible soils as to the amount applied.

As a working principle it is suggested that a *p*H of 6 is sufficiently high for the needs of practically all crops on any soil. If below this, it is evident that only such an amount of lime should be given as a dressing which will raise the *p*H to this figure, at any rate on susceptible soils. Not only must the quantity be controlled but the type of lime used and the method of application are points equally important. It is suggested that a ground form of lime (either carbonate or oxide) should be used, one-half applied before and one-half after ploughing, both applications being thoroughly worked into the soil. Only by some such method would it be possible to obtain really adequate mixing and to avoid the excessive alkalinity due to localized effects which may otherwise result. This problem is particularly urgent at the present time with the widespread increase in the use of lime being made under the stimulus of the Land Fertility Scheme. While it is particularly important that nothing should be said

which will in any way curtail the effort at present being made, a word of warning to the farmer to exercise care under certain circumstances is certainly necessary.

In the West Midlands, as indeed elsewhere, very considerable areas of black soils occur and on these care is being exercised in the amount recommended in all cases where fields are examined and samples taken by responsible persons.

In those areas where susceptible soils are prevalent, doubtless, as in other areas, many farmers apply lime without recourse to sampling. These are the difficult cases, since the farmers by their own zeal may ultimately injure their crops.

Too much caution cannot be exercised by all those who act in advisory capacities, particularly with respect to recommendations of lime on soils wherein manganese deficiency may develop.

(The paper was illustrated by lantern slides.)

II. THE EFFECT OF BORON ON THE GROWTH AND QUALITY OF SUGAR BEET

By A. W. GREENHILL, PH.D., M.Sc., F.I.C., A.R.C.S.

Director, Boron Agricultural Bureau, London, S.W. 1

IT is now recognized that the presence of a small amount of boron is essential for the healthy growth of the beet plant. This was first discovered, about eight years ago, by the German scientist Brandenburg, who grew beets in water cultures. In the absence of boron the plants remained backward in growth and developed certain disease symptoms, the characteristic feature of which was the withering and death of the heart leaves. The plants to which boron was supplied were completely healthy. Brandenburg further showed that a small supply of the element is required by the beet plant at all periods of its growth. His findings have been confirmed in this country by Rowe at Rothamsted, and by workers in other countries. Rowe found that the apical meristem of the shoot, the youngest leaves and the newly developed cambiums of the beet are the parts most sensitive to boron deficiency, and the first to degenerate. Hypertrophy of the cambial cells and of the adjacent parenchyma cells, together with necrosis and disintegration of the phloem, characterize the later stages of deficiency. Evidence was obtained that the first indication of boron deficiency is plugging of the sieve-tubes.

Brandenburg extended his investigations to the field. A disease of sugar beet, in which the heart leaves withered and died, had been known commonly in practice for many years, though its cause had remained obscure. He treated some of the soils upon which the disease occurred with a small quantity of borax or boric acid, and found that it controlled the disorder. His discovery led rapidly to the conduct of many similar and equally successful experiments in the chief European countries, including the British Isles, and in the sugar beet growing districts of the United States.

Symptoms of Heart Rot disease

The disease is known generally as Heart (or Crown) Rot. It becomes apparent usually in late July or in August or September, following a period of dry weather. The first visible symptoms are seen always in the *youngest or heart* leaves, which cease to

grow and become curled. Dark brown patches appear on their stalks, chiefly on the inner sides, and the stalks themselves are found to be abnormally brittle. Within a short time, the heart leaves wilt and turn yellow and soon blacken and die: the main growing point is killed. The older, outer leaves may become affected in the same way later, especially if the dry weather continues. In severe cases all the leaves may die. Sometimes, and more particularly with the onset of rains, groups of young leaves develop from secondary growing points, giving the plant a characteristic short, green top and often tending to mask the original symptoms. These leaves may also become affected later.

The symptoms shown by the leaves are followed generally by a characteristic discoloration and rot in the root, to which the name Dry Rot is usually given. The crown becomes brownish or black in colour. Depressed spots develop and penetrate into the flesh in the form of a Dry Rot. These spots gradually increase in size and in badly affected plants may bring about the destruction of a large part of the root. In some cases, also, internal brown streaks spreading down the root may be seen on cutting it open longitudinally.

Effect of disease on yield and sugar content

Not only does Heart Rot reduce the yield per acre; the disease is also accompanied by great changes in the metabolism of the plant, affected beets containing a lower percentage of sugar than healthy ones. Thus there is a double loss, and the reduced monetary return from an affected crop is often very considerable. In a severe attack in Norfolk investigated by Hanley & Mann in 1935, the yield from a 16-acre field, on the factory returns, was only 2·3 tons of washed beet per acre. The average sugar content was $14·3\%$, and a trial lifting of the more severely affected beets showed a value of only $8·1\%$. The danger of such roots being included in the sample for analysis at the factory will be appreciated. In another case in Norfolk, which the same workers investigated, the presence of the disease reduced the yield of sugar per acre by over 30%. An average reduction of 38% in the yield of sugar was reported for the same year by the Department of Agriculture, for twenty centres on which the disease occurred in the Irish Free State. The serious nature of such losses needs no emphasis.

Influence of soil and weather conditions on Heart Rot

Heart Rot occurs to a varying extent in practically all beet-growing countries, including the British Isles. The annual loss to farmers in Poland through the disease, for instance, has been given as about the equivalent of £300,000 sterling. In Germany, it is estimated that somewhere between 150,000 and 200,000 acres of sugar beet were treated with boron in each of the past two seasons. Whilst the disease occurs on a variety of soils, it is most prevalent on the lighter types. It may, however, appear on the heavier soils, as instanced by the attack reported in 1935 at Rothamsted.

The disease is most frequently associated with alkaline conditions in the soil. Attacks on acid soils have, however, been recorded. The effect of heavy applications of lime in inducing the appearance of the disease has now become widely recognized, and several official warnings have lately been given of this possible danger from over-liming.

The soil moisture content appears also to play an important part in determining the incidence of Heart Rot. The disease is associated mainly with a low soil moisture

content, and so with dry summers. The conditions most conducive to its occurrence appear to be a wet spring, in which growth is rapid and unchecked, followed by hot, dry conditions in July and August. Soils bearing an affected crop in a dry year may, in a wet season, produce a crop which is relatively or completely free of Heart Rot. The disease often occurs in patches in a field. In the case of sloping or undulating land, it will usually be more noticeable on the higher, drier parts. Generally speaking, the largest plants in a field are likely to be the first to be affected.

Heart Rot due to boron deficiency

It is of interest to record the earlier views on the cause of Heart Rot, and the modification of these following the investigations of Brandenburg and others in recent years. The early investigators noticed that the fungus *Phoma Betae* was generally present in the diseased tissues of affected beets. They concluded that this fungus was the direct cause of the disease. It became recognized, however, that *Phoma Betae* was a weak parasite, able to attack only beets whose powers of resistance had been lowered in some way. We now know that where Heart Rot is accompanied by *Phoma Betae* attack, the latter is of a secondary nature. Subsequently, the view came to be held that Heart Rot was due to physiological disturbances, associated usually with alkaline soil conditions.

From the evidence available, it is now generally accepted that Heart Rot is due to a deficiency of boron. This deficiency may occasionally be a primary one. In the majority of cases, however, it is probably induced by alkaline soil conditions rendering the boron naturally present in the soil unavailable to the plant. This effect is most marked under conditions of low soil moisture content, and on soils of low water-retaining capacity. Whether it is due to direct chemical reaction, or to biological factors, or to both, is still uncertain.

Several observations made recently further confirming the present view are worthy of mention. Brenchley & Watson, following the attack of Heart Rot at Rothamsted in 1935, collected some diseased roots from the field, and transplanted them into sand cultures. Boron was added to some of the cultures and omitted from others. Except where the disease had already done irremediable damage before transplanting, the boron-treated plants all produced healthy shoots. The plants which received no boron, on the other hand, produced shoots showing the characteristic signs of deficiency. Cook, at the Michigan Agricultural Experiment Station, U.S.A., has confirmed the findings of Brenchley & Watson, by cutting diseased roots into halves and growing the halves in culture solutions containing boron and without boron respectively.

The view that Heart Rot is due to a deficiency of boron was criticized earlier on the ground that healthy roots and those suffering from Heart Rot contain similar amounts of boron. Recently, Brandenburg has shown that although this is so, large differences exist between the boron contents of the leaves of healthy and diseased plants. He obtained the following figures in 1935 and 1936:

	mg. H_3BO_3/kg. dry matter	
	1935	1936
Leaves of diseased plants	93	72
Leaves of healthy plants from the same field	202	161
Leaves of healthy plants from fields free from Heart Rot	220	236

Brandenburg states: "The boron taken up by the beet is for the most part fixed in the leaves, and can be further mobilized only with great difficulty....The leaves formed during a deficiency period have an extremely low boron content, whilst the outer leaves still show the normal amount."

Boron treatment of Heart Rot

It would be expected that any factor which has the effect of increasing the amount of available boron in the soil would tend to reduce the incidence of Heart Rot. We see this in the influence of wet summers when the soil moisture content is maintained at a relatively high level. Also, it has been shown that the addition to the soil of materials which reduce soil alkalinity, lessens the incidence of the disease.

In general practice, however, the best method of treatment has been found to be the application to the soil of from 15 to 20 lb. of borax per acre. This should be applied preferably when preparing the land for sowing, but it can be given during the early growing period, or even later when the first symptoms of disease appear. This late application, however, is usually less effective than is an earlier one. To apply satisfactorily the small quantity required, the borax may be incorporated in the ordinary fertilizer mixture, or it may be given as a separate application after admixture with a spreader to provide sufficient bulk for even distribution. In view of the known toxic effects of excess amounts of boron, uniform mixing of the borax with the other materials, and even application over the land, are important. Further details concerning the application of borax to soils, and its admixture with other fertilizers, are given in various publications on the subject.

In practically all of the many experiments which have been conducted in recent years in a number of countries, boron treatment of the soil has completely, or almost completely, eliminated the symptoms of Heart Rot. At the same time there has been a considerable increase in the yields of roots and tops, and in the percentage sugar content of the roots. Some typical results obtained in experiments conducted in this country and in the Irish Free State are shown in Tables I and II respectively.

Table I. *Effect of borax on sugar beet in Norfolk, 1935**

Borax applied lb. acre	Beets showing Heart Rot %	Yield of washed beet tons acre	Sugar content %	Yield of sugar		Weight of tops tons acre
				cwt. acre	% increase	
0	51·2	5·7	14·4	16·5	—	2·3
4	42·5	6·2	14·9	18·4	12	3·4
14	6·1	7·1	15·9	22·5	36	4·1
28	5·9	7·5	16·2	24·1	47	4·3

* Hanley, F. & Mann, J. C. (1936). The control of Heart Rot in sugar beet. *J. Minist. Agric., Lond.,* **43**, 15–23.

The application of borax to beet crops at rates above 20 lb./acre is not usually necessary, and, in view of the toxic effects of excess amounts, is not generally to be recommended. It is of interest to note, however, that at the Norfolk Agricultural Station, in the seasons of 1936 and 1937, applications up to 1 cwt./acre produced no apparent harmful effects on sugar beet or the following crop. The number of such tests which have been carried out is, however, small, and there is evidence that similar results would not obtain on all types of soils.

Table II. *Effect of borax on sugar beet in Irish Free State,* 1935*
(Average returns from 20 centres)

Borax applied lb./acre	Incidence of Heart Rot	Yield of roots (net factory wt.) tons/acre	Sugar content %	Yield of sugar	
				cwt./acre	% increase
0	Considerable	8·2	16·6	26·9	—
14	Less extent	11·1	18·1	40·0	49
21	Very small	11·6	18·3	42·3	57
28	Practically free	11·9	18·3	43·6	62

* Crown Rot in sugar beet (1936). *J. Irish Free State Dept. Agric.* 34, 131–2 A.

Unfortunately, there is no ready means at present of examining a soil chemically in the laboratory and saying whether or not it is deficient in boron. If its boron content is determined, the figure can have only a limited value, since a number of factors appear to influence the availability of the element to the plant. The only sure means at present of diagnosing boron deficiency in the soil is the observance of deficiency symptoms in the plant.

In view of the very striking results obtained in the control of Heart Rot by boron treatment, experiments have also been carried out on soils from which the disease was absent. The application of borax under these conditions has been found usually to be without effect on either the yield or the percentage sugar content of the beets. In some cases, a small increase in yield has, however, been obtained.

In the absence of Heart Rot, it is generally assumed that the natural boron content of the soil is adequate for the needs of the beet crop. This is no doubt mostly true. The possible future development of a condition of boron deficiency in more of our sugar beet soils should, however, be kept in mind. The appearance of Heart Rot is almost certainly indicative of an advanced stage of deficiency, and there are probably many soils at the present time in which less acute conditions of deficiency exist. Concerning these we at present know very little. In the past, the boron reserves of the soil have no doubt been maintained in some measure by the use of farmyard manure and some of the older chemical fertilizers, which contain appreciable traces of the element. With the present decreasing use of farmyard manure, and the reduced minor element content of some of the modern chemical fertilizers, those former sources of supply are diminishing.

The question is often asked: How long does a dressing of boron remain effective? From the evidence so far obtained, it appears that the normal dressing of 20 lb. borax/acre may be expected to remain effective for two or three years at least.

The fear has sometimes been expressed that the application of boron to beet soils may in time result in the accumulation of the element in the soil to an extent which may be harmful to other crops less tolerant of boron. The results of leaching experiments carried out recently in Germany showed, however, that a large proportion of the added boron not taken up by the crop is likely to be leached out of the soil by rains, and that no harmful accumulation is likely to occur.

III. SUGAR BEET PESTS

By F. R. Petherbridge, M.A.

School of Agriculture, Cambridge

The Black Bean aphis: Aphis (Doralis) fabae *Scop.*

This aphis has previously been known in this country as *Aphis rumicis* L. Continental workers, however, use the name *Aphis (Doralis) rumicis* L. only for the non-migratory aphis which lives on docks.

We have recently found a black aphis which oviposits on *Rumex* and undoubtedly conforms to the continental description of *Aphis rumicis* L. (D. Hille Ris Lambers, 1934, *Stylops*. 3, 25). This aphis is quite different from the Black Bean aphis which has hitherto been referred to as *Aphis rumicis* L. but should now be called *Aphis fabae* Scop. We suggest therefore that the writers who have mentioned oviposition on *Rumex* are referring to *Aphis rumicis* L., a non-migratory species according to continental authorities, and not to the Black Bean aphis. It is not denied that the latter migrates to *Rumex* as well as to other summer hosts.

At present we have only the oviparous form of the true *Aphis rumicis* and know nothing of its biology other than that it curls the leaves of *Rumex* and oviposits on the dead leaves.

Differences between oviparous forms of *Aphis rumicis* and *A. fabae* are as follows:

(1) *A. rumicis* distinctly larger in length of body, cornicles, etc. Length 2·5 mm. *A. fabae* 1·7 mm.

(2) Skin of *A. rumicis* very strongly reticulated. *A. fabae* slightly.

(3) Hind tibiae of *A. rumicis* with few sensoria but otherwise normal. Hind tibiae of *A. fabae* with numerous sensoria and markedly broader or flattened.

Börner further splits *Doralis fabae* Scop. into *D. euonymi* F., *D. viburni* Scop., *D. philadelphi* Börner and *D. mordvilkoi* Börner, but Ris Lambers says: "With the exception of *D. viburni* Scop. which has apterous males, I should prefer to treat them as forms of *D. fabae* Scop. with small differences in biology."

The attack of this aphis on sugar beet was exceptionally severe in 1938 and caused more damage than previously experienced. We estimate the average loss of crop as being over one ton of sugar beet per acre.

Colonies were first found on *Euonymus europaeus* on 9 April and winged forms on 27 April. They were not found on broad beans in gardens until 21 May, but were noted on sugar beet on 23 May and on the ordinary beet crop on 27 May. Enormous colonies were formed on the sugar beet crops during June and early July but at the end of July they had practically disappeared. Predators, especially ladybird larvae, were exceptionally abundant and were responsible for the early disappearance of the aphides. We were unable to find them on the sugar beet crop after the middle of August, but one sugar beet plant (a bolter) in a field near Norwich was found smothered with aphides on 17 September. No other aphides were found in this field.

Return migrants were first found on *Euonymus europaeus* on 16 September. There were very few males and in the Cambridge district the number of eggs laid was very few.

Observations were made on the primary distribution of this aphis; except in one bean field it was not mainly confined to the headlands.

Experiments were carried out to test the value of the following insecticides in controlling the pest on sugar beet seed: (1) quassia extract; (2) quassia extract plus soft soap; (3) nicotine—soft soap; (4) a proprietary preparation containing pyrethrum, atomized; (5) paraffin, atomized; (6) nicotine dust.

Nicotine dust gave the best control and was considered to be a commercial proposition. One seed grower in Bedfordshire carried out our instructions involving topping badly infested plants and then dusting with 3% nicotine dust. His total expenses on a 10-acre field were £2. 4s. 5d. per acre. His yield was $16\frac{1}{4}$ cwt./acre, which was considered very satisfactory indeed for a drought year like 1938. 3% nicotine dust is effective on warm days. 4% nicotine dust gives better results on colder days.

Our observations on the treatment of the ordinary beet crop for this pest suggest that none of the methods used is an economic proposition. With the machines at present on the market it is difficult to reach the aphides with dust, and there is a great need for machines which will deliver dust in such a way that it will reach the lower surfaces of the leaves. Atomizing with a preparation containing pyrethrum was not satisfactory and occasionally caused scorching, and in one case it actually caused a heart rot.

Wireworms

The important damage caused by this pest occurs in April and May when the sugar beet is in the seedling stages. If a sufficient stand of beet can be obtained, then the crop is usually a satisfactory one because the loss caused later in the season is not serious.

In 1937 experiments were carried out in a Fen field ploughed out of old grass in 1936, and where the beet had followed a chicory crop spoilt by wireworm in 1936. Here, by drilling wheat between the rows of beet at the same time as the drilling of the beet, a satisfactory stand of beet was obtained with seed at the rate of 15 lb. and 20 lb./acre respectively. Even the rows with wheat on one side only gave a fairly good stand. The rest of the field was ploughed up and re-drilled.

The yield of the original sowing was 14 tons $3\frac{1}{4}$ cwt./acre, whereas that of the re-drilled portion was 11 tons $5\frac{3}{4}$ cwt./acre.

In 1938 a number of fields were sown with beet and intersown with wheat directly after ploughed-up grassland. The wireworm population of these fields varied from 2–10/sq. ft. In all cases a satisfactory stand of beet was obtained. In one field with a population of 20 wireworms/sq. ft. the experiment was interfered with by birds.

These experiments suggest that sugar beet may be satisfactory as the first crop after ploughed-up grassland provided the land is suitable for growing beet for other reasons than wireworm.

Beet eelworm (Heterodera schachtii *Schmidt*)

This pest, which attacks both chenopodiaceous and cruciferous crops as well as certain weeds such as dock (*Rumex obtusifolius*), knot weed (*Polygonum aviculare*) and hemp-nettle (*Galeopsis speciosa*), was first found in England in 1934 and has now been found in 96 different fields. Many of these are beet-sick.

The following table shows the distribution:

	No. of fields
Bedfordshire	1
Cambridgeshire	1
Isle of Ely	41
Norfolk	45
*Leicestershire	3
*Lincolnshire	2
†Dorset	3

* Reported by A. Roebuck.   † Reported by W. E. H. Hodson.

American workers suggest that the sugar beet eelworm was introduced into America by means of cysts present in small clods of soil mixed with imported beet seed. We have found cysts containing embryonated eggs in the dirt from imported seed but none of these eggs was viable. We have, however, found viable cysts of the potato strain of *Heterodera schachtii* in dirt from English seed.

The following suggestions have been made to assist in checking this pest:

(1) In fields or smallholdings where beet-sickness has occurred or where the egg count is high, sugar beet, mangolds, beetroot, spinach, turnips and swedes, and if at all possible other cruciferous crops, should be omitted from the rotation *for at least five years*. Before these crops are grown again, the egg count of the soil should be determined and expert opinion obtained.

(2) Where beet eelworms are present and where the egg count is low, the crops mentioned in (1) should be omitted for three years.

(3) Where no beet eelworms are present the above crops should be omitted for two years.

As the eelworms are readily spread from field to field by means of carts, tractors and other farm implements, workers' boots or the legs of horses, sheep and cattle, it is desirable to take special precautions in infected areas.

In the case of smallholdings we suggest that special arrangements should be made to enable them to grow potatoes as a substitute for sugar beet.

We further suggest that certain areas embracing a number of infected fields and also fields not known to be infected, should be scheduled as "sugar beet eelworm infected areas". In these areas sugar beet, mangolds, beetroot, spinach, turnips, swedes and other cruciferous crops should be grown only once in three years or preferably once in four years.

Lantern slides were shown illustrating some of the points of interest of the above and other pests of the sugar beet.

IV. PESTS OF THE SUGAR BEET CROP IN THE MIDLANDS

By A. ROEBUCK, N.D.A.

Midland Agricultural College, Sutton Bonington, Loughborough

CURTIS in his *Farm Insects* quotes Dickson's *Practical Agriculture* as saying that mangolds are not injured by insects or drought. He adds that such appeared to be the case until about 1844 when *Silpha* appeared in Ireland and France. His full list of pests is: (1) *Silpha opaca*, (2) *Cassida nebulosa*, (3) *Phyllotreta nemorum*, (4) *Atomaria*,

(5) *Pegomyia*. That was the position before 1850. The sugar beet crop, as distinct from mangolds, might be said to be a post war introduction. Have we reached normality, if such a thing exists, in the pests which attack it? Looking back over the period from 1922, in the Midlands, one's general impression is that there has been a change. In the first year or two of the crop rabbits and *Pegomyia* were the only pests noted. Nowadays *Pegomyia* has lapsed into insignificance and rabbits are accepted as necessary evils. To-day the general pests such as wireworms, slugs, leatherjackets, etc., when abundant, take toll of the crop, as of all other farm crops. In addition to these there are a few pests, such as *Atomaria* and flea beetles, which attack the crops very severely in certain seasons and then apparently vanish for a number of years. At the present time there hangs over the whole industry the fear of *Heterodera*, which has begun to attack the crops in recent years.

Altogether, we have recorded 24 species of pests doing damage in the Midlands on this crop. In this total wireworms, slugs, flea beetles, etc., are counted as single species only.

It is interesting to notice what the status of Curtis's five species of pests has been on the sugar beet crop:

1. *Silpha* spp. He cites *Silpha opaca* and possibly other species. This has not made any headway. We had records in 1924, 1929 and 1936, but in no case was appreciable damage done. In our case the species is not *S. opaca* but mostly *S. atrata*.

2. *Cassida nebulosa* has even made less progress as a pest. We had *Cassida vittata* and *C. nobilis* eating a few leaves on several crops in 1936, but no assessable damage was done.

3. *Phyllotreta nemorum*. The flea beetle recorded nowadays is *Chaetocnema concinna*. In the Midlands flea beetles caused much damage in 1930 and were serious pests in 1935. In both years probably more damage was done by *Phyllotreta* spp. (*P. undulata* and *P. nemorum*) than by *Chaetocnema*.

4. *Atomaria linearis* is still at times a most serious pest. We have had two bad years and two years of moderate attacks. It was a moderately severe pest in 1925, then bad in 1929, moderate again in 1931 and bad in 1935. Fortunately this pest and flea beetles have only been destructive in the same year once, namely in 1935.

5. *Pegomyia hyoscyami* var. *betae* remained with us more frequently but has not been a serious pest. It was most frequent in 1923, 1924, 1925, 1929 and was local in 1930, 1932 and 1937.

Now let us consider the other pests to-day. *Carabid* beetles have attacked odd crops in different parts of the Advisory Province, especially in the years 1924, 1931 and 1938. The species concerned are *Pterostichus vulgaris*, *Pt. madidus* and *Ophonus pubescens*. Miss Ormerod recorded *Pt. madidus* on mangolds in 1885 near Bishop's Stortford. The damage is characteristic. A hole is bitten from an exposed part of the plant, e.g. the top of a root or a petiole of a lower leaf. Why they should attack crops at all is difficult to say. In this respect they compare with the Silphid beetles. In all cases trapping them by folded sacks soon stops the trouble.

Of *Aphis fabae* it is interesting to read in Curtis that: "During the summer of 1847 the prodigious swarms of aphides (*A. fabae*) which suddenly covered the young shoots and undersides of the leaves of almost every plant, so that the surface was blackened by them, was unprecedented." The year 1938 was a similar season. The aphides appeared to be quite content on any herbaceous plant until the end of July, when they left

them abruptly. In spite of this they did not do much damage except on the southern boundary of the Advisory Province, where crops were often completely defoliated. Everywhere, however, they were a constant source of anxiety. *A. fabae* first appeared as a pest in 1929, then again in 1931 and 1937. There were some slight late attacks in 1930. Early attacks, which soon disappeared, occurred in 1933 and 1935.

Chafer larvae (*Melolontha*) have an interesting record in Nottinghamshire and Lincolnshire. They have appeared as pests at 4-year intervals, namely 1928, 1932 and 1936. In 1930 there were one or two attacks and in 1938 two fields were attacked, one in Nottinghamshire by 3-year-old larvae and one in Lincolnshire by 2-year-old larvae.

Plusia gamma has also an interesting record but is of less importance. To Curtis it was a turnip pest and to Miss Ormerod it was the beet moth. She instanced a great attack by these caterpillars in 1879. In this Advisory Province it appeared in fair numbers in 1933, especially in Lincolnshire. In 1936 the caterpillars appeared all over the Advisory Province on every crop, making large holes in the leaves. In Derbyshire, out of the sugar beet range, the swedes were attacked.

Of the general pests, *Cutworms* (*Agrotis* spp.) have been the most troublesome. They appeared in 1924, 1929, 1931, 1932 and 1935. They have been very puzzling the last two seasons. They were abundant in the fields during the autumn, but they did not attack the beets.

Millipedes are a source of anxiety. In some fields they persistently remain and destroy the germinating seeds, in others they nibble the young roots.

Leatherjackets and *slugs* have varied much in abundance from year to year. The former have attacked crops in four seasons and the latter in two seasons during the period under review.

Heterodera schachtii has appeared at five centres:

(a) It was first recorded in the Midlands in 1936. On the same field, in the Isle of Axholme, sugar beet, mangolds and swedes were all attacked. Sugar beet had only been grown on part of this field once before, namely in 1935; red beet had been very frequently grown.

(b) In 1936 a field of swedes near Barton-on-Humber was attacked by *Heterodera*. There is no record of mangolds or sugar beet ever having been grown in this field.

(c) In 1937, swedes in a garden near Lincoln were attacked. In neither of these two cases would the eelworm attack sugar beet.

(d) In 1938 the eelworm appeared at Loughborough, attacking mangolds and sugar beet. It was ultimately found on cabbages after a long search, but they did not seem to be affected by the parasite.

(e) During this winter the cysts were found in a field in Derbyshire near Burton-on-Trent, which had just grown a crop of sugar beet. They were very sickly and could not have fed on the beet but probably on the various *Brassica* crops which had been grown in previous years.

It would seem that there is every gradation in these cases between a wholly *Brassica* strain and a strain which attacks beet and *Brassica* spp. equally.

Another eelworm appeared in November 1936, namely *Anguillulina dipsaci*. This appears on the maturing beets and produces a characteristic powdery corky canker round the crown. It is widespread but the losses are not great because of the lateness of its appearance. This is probably the same strain which attacks potatoes at uncertain intervals.

V. SUGAR BEET DISEASES

By H. H. STIRRUP, M.Sc.

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INSTEAD of giving a set paper, I propose to show about 50 lantern slides illustrating various sugar beet diseases and to comment upon them.

Most of the troubles that affect sugar beet in the seedling stage are grouped together by farmers and others and regarded as a single disease, this being called Black Leg. Used in this way, the term really covers a number of troubles, due to different causes. The first few slides illustrate the symptoms of the following seedling troubles: (1) True Black Leg, (2) insect injury, (3) damage due to soil acidity and (4) wind damage.

The first essential for healthy seedling growth is a good seed-bed. Cloddy seed-beds result in weakly seedlings that are unable to withstand the attacks of various parasitic fungi; the result is the loss of many seedlings from various causes.

True Black Leg is caused by certain fungi attacking the hypocotyls of seedlings growing in unfavourable conditions. The most important of these fungi is the seed-borne *Phoma Betae*, but species of *Pythium* and *Rhizoctonia*, which are soil inhabitants, are also concerned. The following slides show the symptoms of varying degrees of attack by the fungi that cause Black Leg.

The chief adverse growing conditions which render seedlings liable to attack by these fungi are: (1) poor tilth in the seed-bed, (2) low soil temperature, (3) shortage of soil moisture, and (4) cold winds. Numerous experiments have shown that seed treatment using an organic mercury compound exercises a certain amount of control of Black Leg when the seedlings are growing under adverse conditions, and also increases the number of seedlings emerging through the soil. When, however, soil and weather conditions are favourable for germination and young seedling growth, little or no benefit accrues from seed treatment.

There are two distinct types of injury to seedlings associated with soil acidity. (Slides illustrating the symptoms of these troubles.) In the first type, the damage is confined to the radicle and young lateral roots, the hypocotyl remaining unaffected. The root system becomes tough and string-like and this type of acid soil injury has been provisionally named "Stringy root". The second type of injury occurs on the hypocotyl only; instead of increasing in size in the normal manner, the hypocotyl remains thin and tough and its surface becomes brown and scurfy. This type of injury is provisionally named "Stringy hypocotyl" and at certain stages it is almost indistinguishable from Black Leg. Various fungi, chiefly species of *Fusarium*, have been isolated from the roots and hypocotyls of seedlings with acid soil injury and it seems likely that these weakly parasitic fungi are able to attack the underground parts of the plants only when they are weakened by growing under adverse acid soil conditions.

Strong winds can cause extensive damage to sugar beet seedlings, particularly on light, sandy soils and also on certain peaty soils which, when dry, assume a texture like that of fine ashes. Soils of this type are easily "blown". The damage may be done to the hypocotyls, cotyledons or true leaves. (Slides.) When the hypocotyls are affected, they become thin and shrivelled, and this type of injury is thought to be one of the early stages of Strangle disease. (Slides showing various stages of this latter

trouble.) It is not caused by insects, as was formerly thought. It is true that insects are attracted to the strangled part of the hypocotyl by the sugary exudate just before, or just after, the top of the plant breaks off, but such insects are merely secondary. Although there is no definite proof yet, it is thought that Strangle disease originates in the seedling stage as some form of internal injury to the hypocotyl near soil level. The formation of the constriction round the hypocotyl in that region is the result of rapid growth and increase in girth above and below the injury, but not immediately underneath it. Subsequently, if weather conditions are favourable for the rapid growth of the plants, they become top heavy and break off at the strangled region. Observations point to the fact that (a) acid soil injury and (b) wind damage, particularly when the seedlings are growing in a soil with a firm, crusty surface, may be two of the primary causes of Strangle disease.

Downy Mildew, caused by *Peronospora Schachtii*, has become much commoner on sugar beet in the last ten years and this disease is capable of causing considerable damage to both root and seed crops. Every year there is a cycle of infection from the seed to the root crops and back again to the young seed plants. Experiments are being carried out to find a method of breaking this infective cycle by spraying young plants in the seed-beds to prevent, or reduce the amount of, initial infection. One of the next seven slides shows a final stage of Downy Mildew attack, the thickened and distorted leaves having become a brown, shrivelled and desiccated mass; at this stage, it is very difficult to distinguish Downy Mildew from Heart Rot, the boron deficiency disease.

Of the four virus diseases of sugar beet known to occur in different parts of the world, Mosaic is the only one that has been recorded with certainty as occurring in this country. Of the non-virus types of Yellows, Crackly Yellows is by far the most important. The coloured photographs (slides) show all stages in the development of this trouble from the primary yellowing of the older, outer leaves to the final stages when the moribund parts of the leaves become invaded by various weakly parasitic fungi (chiefly *Alternaria* spp.) resulting in the so-called Leaf Scorch condition. The next two coloured slides show a different final stage of Crackly Yellows, called Red Leaf. The exact cause of this complex disease known as Crackly Yellows is not yet known, but it seems highly probable that shortage of soil moisture and nitrogen starvation are two of the factors involved.

I propose now to illustrate Speckled Yellows (slides), the manganese deficiency disease, and Heart Rot, the boron deficiency trouble, but in view of the papers read this morning I shall not comment on these diseases.

Rust, caused by *Uromyces Betae*, is not a serious disease of sugar beet in this country. It occurs most frequently in coastal regions and is usually most severe on good, well-grown crops. The two coloured slides illustrating this disease are particularly interesting because they show the cluster cup stage of the fungus, a rarity on sugar beet in this country.

The coloured slide of *Cercospora* Leaf Spot shows a mild attack of this disease. Although comparatively unimportant in this country, this Leaf Spot is a very serious disease in certain continental countries and regular spraying operations have to be carried out there to control it. A high temperature and high atmospheric humidity are the necessary conditions before this disease can assume epidemic proportions.

The most serious root disease of sugar beet is Violet Root Rot (*Helicobasidium purpureum*). The two coloured slides show the symptoms on (a) a root just pulled

out of the ground with the mass of soil adhering to it and (*b*) a root from which the clinging soil has been washed, together with a section of a diseased root to show the depth to which the rot has penetrated.

The next slides show the symptoms of various other root rots, including (1) the black, shiny rot caused by *Phoma Betae* on a root not affected with Heart Rot, the boron deficiency trouble; (2) Wet Rot, caused by *Phytophthora megasperma*, a disease that occurs only in heavy soils that are unduly wet; (3) Internal Zoned Rot, a disease of unknown origin in which the internal tissues of the root contain well-defined zones of necrotic tissue; it has not been possible to isolate any parasitic fungi or bacteria from the affected tissues and it is considered that this disease is a physiological one; (4) Eelworm Canker, caused by *Anguillulina dipsaci*, already referred to in a previous paper.

The next two slides show the symptoms of lightning injury on sugar beet roots, the first on comparatively young plants and the second on older ones. The internal cavities can be clearly seen, and in the older root the point of entrance of the charge is visible. When lightning strikes a field of beet, the affected patch is roughly circular in shape and rather small, rarely more than 10 yards in diameter.

Finally, the last coloured slide shows the effect of spray injury on a sugar beet plant. In the early stages, the symptoms are very similar to those of Heart Rot, the boron deficiency disease, but later a secondary rot usually develops at the injured crowns. This type of injury occurred for the first time last season in a crop that had been sprayed with pyrethrum extract against aphis attack. It is considered that the damage was not caused by the pyrethrum but by the unsuitable oil base of the spray.

REVIEWS

Wood Pulp. By JULIUS GRANT. Pp. 209. Leiden, Holland: Chronica
Botanica Co. (London: Wm Dawson and Sons.) 1938. Guilders 7.

This book is intended to cater for those interested in plant science, and for
students and general readers, but how far it will succeed in doing this is somewhat
uncertain.

The work is divided into 20 chapters commencing with a general introduction in
which wood pulp is defined and its relationship to other fibrous materials discussed.
Thereafter follows a brief historical sketch of paper and pulp manufacture. Chs. III
and IV, which deal with cellulose and the wood fibre, and with the identification and
evaluation of pulping woods, and which should, therefore, be of direct interest to
workers in plant science, are by far the weakest chapters in the book. The section on
the chemistry of cellulose is far from being well done, and a certain looseness of
expression tends at times to confuse the reader. For instance, it is difficult to reconcile
the statement on p. 24 that the term "cellulose" does not signify a definite chemical
entity, with a subsequent section on the molecular structure of cellulose; the more
especially since no indication is given that the structure in question was established
for the alpha cellulose of the cotton seed hair and not for wood cellulose. The few but
none the less important papers on the constitution of wood cellulose are not dealt
with. Free use is made of the term "compound cellulose" which was of more signifi-
cance a generation ago than it is to-day. Most authorities would agree that to apply
this term to hemicelluloses and particularly to holocellulose is a complete mis-
representation of modern opinion. Botanists will hardly be favourably impressed by
the Section on the structure and growth of trees. Having stated early in Ch. IV that it
is seldom necessary to identify a particular wood before it is pulped, the author
proceeds to deal with the subject in some detail. An unfortunate misuse of the terms
"hardwood" and "softwood" on p. 36 leads to confusion throughout the book wherever
they are mentioned; tyloses are described as distended vessels. After stating that
specific gravity varies from one type of wood to another the author invites comparison
between cork and hardwoods in this respect. Several obvious mistakes are made in
describing the determination of lignin in wood. It is stated that xylan is present in
the cell walls of most trees except conifers. These and other examples which could be
cited serve to show that the author is by no means at home with the botany and
chemistry of his starting material. In describing the purely technical side of his
subject he is obviously in known territory and a tolerably readable account is given
of the mechanical, sulphite, soda and sulphate processes, but even here his incursions
into the chemistry connected with the processes are marked by inaccuracies and con-
fusion of terms. Semi-chemical pulping is not discussed but bleaching is dealt with
in some detail. The chapters dealing with the by-products of the chemical pulping
processes are informative. Representative lists are given of the tests applied to the
finished pulp. The three short concluding chapters on the uses of wood pulp are among
the best in the book.

<div align="right">W. G. CAMPBELL.</div>

Manual for the Determination of Seed-Borne Diseases. By L. C. DOYER.
Pp. 59, plates 33. Wageningen: International Seed Testing Associa-
tion. 1938. Fl. 5.

This work has been compiled by Dr Lucie C. Doyer, Mycologist of the State's Seed
Testing Station, Wageningen, in collaboration with the "Committee for the deter-
mination of seed-borne diseases". The booklet is in three parts: (1) a general section
containing a brief classification of the conditions and germination behaviour of

contaminated or diseased seed, and an outline of methods of investigation; (2) a special section in which are discussed infections by fungi and bacteria and infestations by insects and nematodes in seeds of cereals, grasses, peas, beans and clover, beet, flax, cabbage, celery and parsley, carrot, spinach, lettuce, onion, salsify, tomato, corn-salad, and trees; the section closes with a note on saprophytic fungi present on seeds; (3) a tabulation of seed-borne infections and infestations arranged in parallel columns under headings according to method of determination.

The plates contain line, half-tone or colour illustrations of diseased seeds and the organisms parasitic upon them, and are loose in annotated folders.

The work is not a complete manual of seed-borne diseases but it includes most of the more common troubles and it is a very useful publication of unusual and pleasant format.

WILLIAM B. BRIERLEY.

Fungi of India. Supplement I. By B. B. MUNDKUR. Pp. iii + 54. Scientific Monograph No. 12, Imperial Council of Agricultural Research. Delhi: Manager of Publications. 1938. 2s. 3d.

Butler and Bisby's *Fungi of India* (1931) recorded 2351 species, exclusive of the Myxomycetes. The present work adds 79 Myxomycetes, and 433 Eumycetes distributed as follows: Phycomycetes 54, Ascomycetes 67, Ustilaginales 6, Uredinales 66, Autobasidiomycetes 62, Fungi Imperfecti 178. There is one new species, *Mycosphaerella Tinosporae*, with description and Latin diagnosis, and six new combinations. Interesting notes are appended to most of the new records. There is a useful bibliography of 134 citations, and an index of genera.

WILLIAM B. BRIERLEY.

The Vegetables of New York. Vol. I. By U. P. HEDRICK. (Part I. Peas of New York. Pp. vi + 132. Plates 24. 1928. Part II. Beans of New York. Pp. iii + 110. Plates 39. 1931. Part III. Sweet Corn. Pp. iv + 111. Plates 24. 1934. Part IV. The Cucurbits. Pp. iv + 131. Plates 49. 1937.) Albany, N.Y.: J. B. Lyon Co.

The magnificent volumes on tree and small fruits published by the New York State Agricultural Experiment Station under the general authorship of Dr U. P. Hedrick have long been familiar landmarks in horticulture. In 1925 a similar work was planned to deal in three volumes with the vegetables of New York, and the first volume has now been completed. The second volume will deal with salad crops and pot herbs, and the third with cole and root crops. Although the title relates the work specifically to the State of New York, the contents are more catholic and almost inclusive of North American vegetables.

The four parts of vol. I have been issued separately at intervals of a few years but, in each, a more or less standard plan is followed: History of the plants; systematic botany; detailed description of varieties; bibliography and index. It is not easy to do justice to the almost epic scale upon which the work is planned, the high quality which is sustained throughout, the fullness of treatment, or the accuracy of detail. Owing to the number of different plants to be considered a discussion of their culture, or of their botany, much of which is in need of investigation, would have enlarged the volume beyond necessity, but on such aspects as are considered the work is encyclopaedic. The historical chapters are fascinating, containing a wealth of allusion to early scientific and literary sources. The main portion of the work is concerned, naturally, with descriptions of varieties the selection of which has been guided partly by their horticultural and economic status, partly by their importance in plant breeding, and

partly by their value in illustrating systematic relationships and evolutionary trends. The actual descriptions are excellent: original and based on living specimens, and giving tersely yet fully an idea of all the characters of the several varieties. The style of writing is of high quality, simple, clear and condensed, yet interesting, and the beautiful printing, in double column on the page, makes the text easy to read. Lastly there are the illustrations, which are a sheer joy: full-page colour photographs, splendidly reproduced, and something almost unique even in horticultural literature.

In part I collaborating authors were F. H. Hall, L. R. Hawthorn and Alwin Berger; and in parts II–IV, W. T. Tapley, W. D. Enzie and G. P. van Eseltine.

<div align="right">WILLIAM B. BRIERLEY.</div>

Cotton: History, Species, Varieties, Morphology, Breeding, Culture. Diseases, Marketing, and Uses. By H. B. Brown. 2nd ed. Pp. xiii + 592. London: McGraw-Hill Publishing Co.. Ltd. 1938. 30s. 0d.

The first edition of this book received notice in the *Annals*, 1927, **14**, 564. In the new edition, which is larger by 75 pages, the general plan and chapter headings remain unchanged but, in every chapter, there have been considerable emendation, rewriting and addition of new paragraphs to bring the work up to date. Many of the tables have been amplified, the statistical tables usually include 1935 or 1936 data, and more recent references have been added to the bibliographies. Several new text-figures have been inserted and some of the earlier figures have been changed to illustrate later developments.

In spite of these alterations the book remains almost purely American in outlook; e.g. of the additional references in the new edition 80°₀ are to U.S.A. publications. So far as it goes the book is a useful compilation but it might easily have been converted into a standard work.

<div align="right">WILLIAM B. BRIERLEY.</div>

The Longevity of Plants. By H. Molisch. Translated into English by E. H. Fulling. Pp. 226. New York: Published by the Translator. Science Press Printing Co., Pa., U.S.A. 1938. $3.

Molisch's *Die Lebensdauer der Pflanze* (1929) is still the only book on this intriguing subject. The author discusses the longevity of unicellular and multicellular plants, longevity in relation to systematic relationships, organs and tissues, means of prolonging the life of plants, rejuvenescence, apparent death, and old age, death, and the alleged perpetual life of trees.

Although following closely the meaning of the German text, the translation is free and readable. Some of the tables have been rearranged, and some of the original illustrations omitted or replaced by excellent photographs by the U.S. Forest Service. The references, which in the German text were given as footnotes and often cited incompletely, are cited in full and relegated to a bibliography. Publications since 1928 are collated and classified in a second bibliography, and the names of the authors included in the author index. The subject index has been recast.

The writing of a new edition would have involved extensive reconsideration in view of more recent studies on vernalization, photoperiodism, plant and seed dormancy and the breaking of the dormant period, plant hormones, polyploidy, sex reversal, etc., and it is a great pity that this could not have been done since an up-to-date treatise is very desirable. Much of Molisch's work, however, remains valuable, and this excellent translation will serve to focus attention on an interesting and important problem.

<div align="right">WILLIAM B. BRIERLEY</div>

Plant Physiology. By N. A. MAXIMOV. Edited by R. B. HARVEY and A. E. MURNEEK. Second English Edition, translated and revised from the Fifth Russian Edition. Translated from the Russian by Dr Irene V. Krassovsky. London: McGraw-Hill Publishing Co., Ltd. 1938. 25s. 0d.

In preparing this new translation of Maximov's *Text-book of Plant Physiology* the editors have altered the title in order to avoid confusion with the former edition. It would have been even better if they had given it a title which would have indicated its very special character. The author himself in his preface to the Russian edition explains that his objective was to present "to the students of our universities and agricultural colleges and to our future technical agriculturists clear and exact information concerning the life and vital functions of green plants". The whole tone of the book is towards what has been termed "agrophysiology". It marks an entirely new departure in works on plant physiology; the plant is no longer considered as an organism to be analysed according to its separate functions but as a unit "in which all the parts and all the processes taking place in them are closely inter-related". It is the first time that what may be called the dynamic viewpoint, in contrast to the static outlook of the classical, mechanistic physiologists, has directed the writing of a text-book. The whole conception and arrangement of the volume is different from the usual standardized form.

The approach is first to the physico-chemical foundations of the plant and its mechanisms of metabolism. The discussion then passes, surprisingly though logically, to the earliest functions of the germinating seed, respiration and growth. The synthetic and absorptive processes are next reviewed, followed by chapters on the water relations of plants and the translocation of substances through the tissues. Ch. x is a masterly discussion of the resistance of plants to unfavourable environmental conditions, a subject on which the author is the primary authority. The remaining chapters are concerned with growth-correlations, the physiology of development, flowering and fruiting, and seasonal phenomena in the life of plants. In these chapters all the modern applications of physiology to agriculture and horticulture, including the hormone theory, vernalization, photoperiodism, fruit-ripening and so on, are reviewed more adequately than has ever before been done in such a book. Throughout, the outlook is on the plant as a dynamic organism, not a static thing to be analysed into its component parts and processes but a correlated whole whose parts and functions are mutually interdependent.

The editors have carried out their task in complete sympathy with the author, while at the same time introducing their own viewpoints. The book is not merely a translation from the Russian but has been widely modified to make it of more widespread interest. New illustrations and data have been used and references have been reselected from publications in English.

It would mean a revolution in our botanical schools if this could be taken as the standard work on physiology, but the gain would be immense. The new outlook is gaining ground in all the schools of applied biology; if it can invade the strongholds of the departments of pure science the mechanistic foundations will begin to crumble at last.

R. H. STOUGHTON.

Science in Agriculture. By J. W. PATERSON. Pp. viii + 288. 165 illustrations. London: Longmans, Green and Co., Ltd. 1938. 6s. 6d. net.

This book does not attempt to teach farming, but deals with fundamental principles in a way likely to provide a healthy incentive to consider problems from the more practical aspect. It will be particularly helpful in schools where science with a rural bias is incorporated in the syllabus. To teachers who have to deal with this

subject, and more particularly to those who are town bred and have not previously studied agriculture, the book will make a strong appeal. It should give them that confidence in their subject which is so essential to success in imparting knowledge to others.

The author suggests that there is little reason why pupils in secondary schools should not receive their mental training from the study of Agricultural Science instead of the more orthodox chemistry, physics or biology, and his book goes far to make this possible. Its arrangement into numerous chapters, each dealing concisely with a clearly defined subject, makes it a handy book of reference to the farm pupil who wishes readily to gain information on a specific point. Some of the material may be a little too compressed, and a better balance might have obtained between the space devoted to crops as opposed to stock. One notable omission is that no reference is made to poultry, which not only make ideal subjects for practical school instruction purposes, but must now be considered as an important class of farm livestock. The book is written in a clear attractive style, and is well illustrated by up-to-date photographs and excellent sketches. It is of a handy size, and the printing is admirable, good use being made of heavy type and italics.

<div style="text-align: right">D. J. G. BLACK.</div>

Report on Agricultural Research in Great Britain: a Survey of its Scope, Administrative Structure and Finance, and of the Methods of making its Results known to Farmers, with Proposals for Future Development. Pp. vi + 146. London: P.E.P. 1938. 8s. 6d. net.

P.E.P. (Political and Economic Planning) is an independent non-party group of trained enquirers recruited from various professions, who devote part of their spare time to fact-finding and to suggesting principles and possible advances over a wide range of social and economic activities. Many of us are familiar with its fortnightly broadsheet "Planning", but from time to time P.E.P. issues full-scale Reports which are of permanent and constructive value.

The agricultural research organization in this country, born under 30 years ago, has developed so rapidly and branched out in so many different directions that, unless one is prepared to wade through numerous Government publications, it is not easy to form a mental picture of its structure and relations. This P.E.P. Report gives exactly such a picture, together with a critical appraisal. It is not concerned with the actual content of agricultural research but with its quality and quantity, the adequacy of the provision made for it, the efficiency and economy of its administration, and to what extent the present machinery for getting the results of research across to the farmer is successful.

The volume opens with a brief summary of the aims of the Report and of its findings and closes with six appendices listing institutions for agricultural education and research, and data relative to their expenditure and staffing, and a final appendix briefly outlining fishery and forestry research in Great Britain. The factual text of the Report is arranged in seven chapters of which the first is introductory. Ch. II surveys the structure of agricultural research in Great Britain and the relations of the multiplicity of governing and directing bodies, research and advisory organizations, and information bureaux. Ch. III describes briefly the finance of agricultural research and the various sources of money. Ch. IV outlines the personnel of agricultural research—numbers and subject distribution, grades and salaries, recruitment, etc. Ch. V gives an account of the ways in which scientific knowledge about agriculture is disseminated, and pointedly criticizes some of the rather glaring defects. Ch. VI contains a brief summary, based essentially on the last biennial Report of the A.R.C. (Agricultural Research Council), of what is actually being done in agricultural research —in short, whether an annual expenditure of some £700,000 of public money is being justified. Ch. VII is a brief statement, for purposes of comparison, of the general

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1938

THE Officers and Council of the Association were as follows:

President: C. T. GIMINGHAM, B.Sc., F.I.C.

Vice-Presidents: H. MARTIN, D.Sc., H. WORMALD, D.Sc.

Hon. Treasurer: J. HENDERSON SMITH, M.B., Ch.B.

Hon. Secretaries: General and Botanical: W. P. K. FINDLAY, M.Sc.; Zoological: G. FOX-WILSON.

Hon. Editors of *Annals of Applied Biology:* General and Botanical: Prof. W. B. BRIERLEY, D.Sc.; Zoological: C. T. GIMINGHAM, B.Sc., F.I.C.

Council: H. F. BARNES, M.A., Ph.D., H. A. DADE, A.R.C.S., W. J. DOWSON, M.A., D.Sc., T. GOODEY, D.Sc., H. MARTIN, D.Sc., H. W. MILES, D.Sc., Ph.D., H. C. F. NEWTON, Ph.D., W. C. MOORE, M.A., A. ROEBUCK, N.D.A., E. R. SPEYER, M.A., H. G. THORNTON, D.Sc., H. WORMALD, D.Sc.

The Association has met on six occasions during the year, including two visits. The Annual Summer Meeting was held on 8 July at the Forest Products Research Laboratory, Princes Risborough, and the afternoon visit on 5 November to the Studios of the Gaumont British Instructional Films Co. at Shepherd's Bush, London: to both these institutions the Association is indebted for their kind hospitality.

At ordinary meetings the attendance as recorded in the signature book was, on the average, 39 Members and 18 visitors, an increase on the previous year: 28 Members attended the Summer Meeting, and about 50 the very popular visit to the film studios.

Twenty-one new Members were elected during the year and four Members resigned. The Council wishes to record with regret the death of Prof. Murphy who had been a Member since 1920. The Association now numbers 330 Members including 12 Honorary Members: of the ordinary Members as far as is known, 268 are resident in the British Isles and 50 in the Empire or in foreign countries.

The following papers and discussions were brought before the Association during 1938:

11 February. Presidential address by Dr J. HENDERSON SMITH, *Some Recent Developments in Virus Research.*

18 March. *Discussion on the Use of Chemical Weedkillers.*

M. A. H. TINCKER, M.A., D.Sc.: Weedkillers in Relation to Horticulture.

R. H. MACDOWALL, Dipl. R.T.C., A.M.I.Chem.E.: Some Factors influencing the Agricultural Use of Chemical Weedkillers.

G. E. BLACKMAN, M.A.: The relative Toxicity of Chemical Weedkillers.

R. B. DAWSON, M.Sc., F.L.S.: The Eradication of Weeds in Lawns.

O. OWEN, M.Sc., Ph.D., A.I.C.: Chlorate Weedkillers.

7 October. *Discussion on Freshwater Biology.*

> E. B. WORTHINGTON, M.A., Ph.D.: Introduction: Freshwater Biology and its Applications.

> H. C. MORTIMER, B.Sc., D.Phil.: Chemical Aspects of Organic Production in Freshwater.

> A. C. GARDNER, M.A.: Some Problems of Waterworks Biology.

> Miss M. ROSENBERG, D.Phil.: Algal Physiology and Organic Production.

9 December. *Observation on Apple Canker.*

> R. G. MUNSON, B.Sc.: The Discharge and Germination of Ascospores of *Nectria galligena*.

> R. W. MARSH, M.A.: The Incidence and Control of Canker Infections in Apple Shoots.

> S. O. GARRETT, M.A.: Recent Advances in Biological Control of Soil-borne Fungal Diseases.

The Association during the past year has again enjoyed the privilege of holding its meetings in the Botanical Department of the Imperial College of Science and Technology and in the Metallurgical Lecture Theatre of the Royal School of Mines, and the Council wishes to take this opportunity of expressing, on behalf of the Association, its grateful thanks to the College authorities for the continuation of their valued hospitality.

The Hon. Editors of the *Annals of Applied Biology* report that in 1938 the volume comprised pp. xii + 891 and 37 plates, as against pp. xiv + 940 and 51 plates for 1937, this slight reduction being in accordance with a ruling of Council. Including papers published as "Proceedings", vol. **25** contained 61 papers and 41 reviews, as against 67 papers and 44 reviews for vol. **24**. Of the papers in the 1938 volume, 48 were by Members of the Association and 13 by non-members. The papers may be roughly classified as follows: General applied botany 15; Mycology and fungal diseases 17; Virus and virus diseases 6; General applied zoology 2; Entomology and insect pests 12; Plant protection 8; Helminthology and nematode diseases 1.

The several parts of the *Annals of Applied Biology* were published on the following dates: Part 1, 18 February; Part 2, 27 May; Part 3, 15 August; Part 4, 15 November.

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W. P. K. FINDLAY \
G. FOX-WILSON } *Hon. Secretaries*

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REPORT OF THE HON. TREASURER FOR THE YEAR ENDING 31 DECEMBER 1938

During the year ending 31 December 1938 subscriptions and entrance fees, including arrears paid in, amounted to £324. 13s. 0d., a decline of £13 as compared with last year. Income from the sale of the current volume of the *Annals of Applied Biology* and from reprints amounted to £763. 3s. 0d., a decline of £46 due entirely to a falling-off in the sale of reprints, which is always a fluctuating item. The size of the *Annals* amounted to 891 pages as against 940 in 1937, and the cost of printing amounted to £1255 as against £1330 in the preceding year. In the whole year there has been an excess of expenditure over income of £90. 8s. 0d.

The Association has an excess of assets over liabilities of £762. 10s. 7d. In accordance with the policy of the Council we have again reduced the total of our assets by printing a volume larger than our income covers. We shall not, however, be able prudently to deplete our reserves in this way for more than two or perhaps three more years, and we shall then have to return to a volume of smaller size, such as we used to issue. This will be regrettable, and members can postpone that day in three ways. Firstly, by contributing papers which enhance the reputation and indispensability of the *Annals*: the larger part of our income comes from sales to non-members, and these depend upon the standing in the scientific world of the *Annals*. Secondly, by obtaining new members to more than replace our losses by death and resignation; much might be done in this way, if members would make a little effort. Thirdly, by prompt payment of the subscription: in 1936, the arrears were £37. 10s. 0d., in 1937, they were £60, in 1938 they were £105. This delay reduces our working balance, and so affects the size of volume we can afford to produce.

J. HENDERSON SMITH
Hon. Treasurer

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY,* INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1938 **Cr.**

EXPENDITURE	£ s. d.	£ s. d.	INCOME	£ s. d.	£ s. d.
To Estimated Value of Stock, 1st January 1938		169 19 0	By Sales—Current Volume		638 4 2
To Cambridge University Press		1255 4 10	By Sales—Back Volumes, Parts and Sets		159 12 11
To Copies bought in		15 15 0	By Sales of Reprints		134 18 10
			By Advertisement		1 3 0
			By Estimated Value of Stock, 31st December 1938		150 16 0
			By Balance, carried down		386 3 11
		£1460 18 10			£1460 18 10

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1938 **Cr.**

EXPENDITURE	£ s. d.	£ s. d.	INCOME	£ s. d.	£ s. d.
To *Annals of Applied Biology,* balance brought down		386 3 11	By Members' Subscriptions:		
To Printing and Stationery		9 2 5	Arrears	12 10 0	
To Postages and Cheque Stamps		6 18 11	Entrance Fees	8 8 0	
To Honorarium		5 5 0	Current	303 15 0	324 13 0
To Subscription—Parliamentary Science Committee		10 10 0	By Interest on National Savings Certificates and Bank Deposit		25 4 2
To Sundry Out-of-Pocket Expenses of Secretaries and Treasurer		18 0 11	By Balance, being Excess of Expenditure over Income for the year		90 8 0
To Audit Fee		4 4 0			
		£440 5 2			£440 5 2

BALANCE SHEET, 31 December 1938

LIABILITIES AND SURPLUS	£ s. d.	£ s. d.	ASSETS	£ s. d.	£ s. d.
Sundry Creditors:			Cash:		
Cambridge University Press	347 0 11		At Bank on Current Account	82 6 4	
Audit Fee Reserve	4 4 0		At Bank on Deposit Account	1 0 0	83 6 4
Sundry Expenses	16 19 10	368 4 9	Debtors for Subscriptions, two years or less in arrear and considered good		105 0 0
Subscriptions and Entrance Fees, paid in advance		14 12 0	500 National Savings Certificates		806 5 0
Excess of Assets over Liabilities:			Stock of *Annals of Applied Biology* at estimated value		150 16 0
As Balance Sheet of 31st December 1937	852 18 7				
Less: Balance of Income and Expenditure Account for 1938	90 8 0	762 10 7			
		£1145 7 4			£1145 7 4

A COMPARISON OF THE METABOLISM OF MOSAIC DISEASED POTATOES WITH THAT OF NORMAL POTATOES

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(With 8 Text-figures)

I. INTRODUCTION

THE effects of mosaic diseases upon carbohydrate and nitrogen relations of host plants have received considerable attention. A wide range of plant species affected with many types of mosaic disease have been studied and certain general conclusions may be drawn from the observations recorded in the literature. Most workers are agreed that in actively growing plants mosaic diseases are accompanied by disturbances in carbohydrate metabolism which are revealed in reduced amounts of carbohydrates, particularly of polysaccharides. Starch/soluble-sugar relationships have been found to be chiefly affected and degradation of starch to be retarded. Observations on nitrogen content have shown that nitrogen is usually higher in diseased plants and degradation of nitrogenous materials is affected in similar fashion to degradation of

carbohydrate, i.e. insoluble forms are less readily hydrolysed. In all cases there has been indication of a lower C/N ratio in diseased plants as compared with healthy plants.

The present investigation has confirmed and extended some of these observations. It has also provided results which controvert, in part, and extend those previously published upon the nature of carbohydrate metabolism in normal potato leaves (Davis & Sawyer, 1916; Clements, 1930; Barton Wright & McBain, 1932, 1933a), and it is most convenient, therefore, to present and examine first those data obtained from the healthy material. The findings of this examination will serve, subsequently, as a basis upon which to establish a comparison between the metabolism of mosaic affected leaves and that of normal leaves.

This work formed part of an investigation designed to examine comprehensively, in relation to the continuity of metabolic change, the carbohydrate, nitrogen and mineral metabolism of normal and mosaic diseased potato plants. The investigation had to be curtailed in 1934 before reaching completion, and only those data relative to carbohydrates and total nitrogen in leaves and petioles are available for discussion.

II. Material and methods

The potato varieties used throughout the investigation were President and Arran Victory. The material for each variety consisted of sixty diseased plants, infected with potato virus X and showing symptoms of mild or simple mosaic, and sixty proved virus-free plants to serve as healthy controls. All the plants were grown in a single insect-proof greenhouse, and comparable conditions were maintained between individuals by equality of treatment and frequent change of position. Random samples of twenty whole leaves from the intermediate regions of the stem were taken at intervals of 2 hr. over each of the diurnal periods to be considered. In each case, except that of 20 days old President, the leaves were fully expanded at the time of sampling.

Estimates of the seasonal variations in total carbohydrates and total nitrogen were obtained from samples of twenty leaves removed at weekly intervals from the plants used in diurnal experiments. The weekly samples from Arran Victory were obtained at 10.0 a.m. G.M.T. and those from President at 11.0 a.m. G.M.T.

The treatment of samples in preparation for analysis was similar to that used by Barton Wright & McBain (1933a, b), and drying was carried out in two stages as recommended by Link & Tottingham (1923). This

method has been shown to be satisfactory for both carbohydrates and nitrogen (Pearsall & Wright, 1929).

All carbohydrate analyses were effected by the Schaffer & Hartmann (1921) microtechnique, and total nitrogen was estimated by the method described by Ranker (1925) which includes nitrate-nitrogen in the estimate.

Calculations for diurnal material are based upon the residual dry weight (R.D.W.) (Mason & Maskell, 1928), and the standard error is attached to each calculated mean value. In order to express more clearly the seasonal differences in carbohydrate relationships, the data for each diurnal sample have been calculated also as percentages of the total estimated carbohydrates and subjected to the same treatment as that given to the actual values. Comparisons between mean values have been examined for significance by a calculation of the ratio between difference of the mean and error of the difference, shown in the tables as D/E_D. The odds against D/E_D exceeding 2·80 by accident in a comparison of 24 values are 100 : 1 (see Table of t, Fisher. 1932), so that any value of this ratio greater than 2·80 may be considered indicative of a fully significant difference between the two mean values compared.

III. THE CARBOHYDRATE METABOLISM OF THE NORMAL LEAF

Experimental observations

Diurnal series.

President, 20 days old. The mean values about which diurnal fluctuations of reducing sugars, sucrose and starch occurred in the leaves and petioles of 20 days old President are given in Table I. These data show that reducing sugars predominated in the leaf blade whilst sucrose predominated in the petiole, a result which is contrary to those previously recorded for the potato (Davis & Sawyer. 1916: Clements. 1930: Barton Wright & McBain. 1932) and to the later observations on the present material. Similar high values of reducing sugars were found by Went (1898), however, in those parts of the sugar cane in which rapid extension growth was taking place. The potato leaves sampled at 20 days were not

Table I

Mean values	% R.D.W.	% total carbohydrate	Petiole % R.D.W.
		Lamina	
Reducing sugars	0·973 ± 0·040	44·20 ± 1·46	0·274 ± 0·076
Sucrose	0·376 ± 0·035	16·95 ± 1·36	1·183 ± 0·050
Starch	0·854 ± 0·022	38·91 ± 0·36	0·368 ± 0·060

fully expanded, and there is evidence to suggest that leaves on plants of this age would be developing rapidly through cell differentiation (Stone, 1933) and increasing in weight (Bunzel, 1914). It is not unreasonable, therefore, to interpret the data in Table I as indicative of a metabolism conditioned to support growth activities.

A high reducing sugar content in growing regions was suggested by Went to be linked with the utilization of carbohydrate in growth requirements, since reducing sugars may serve both as substrates in the synthesis of complex carbohydrates and also as the most suitable sugars for the establishment of the high internal pressures necessary to cell extension. Onslow (1931) has accepted and extended these arguments on biochemical
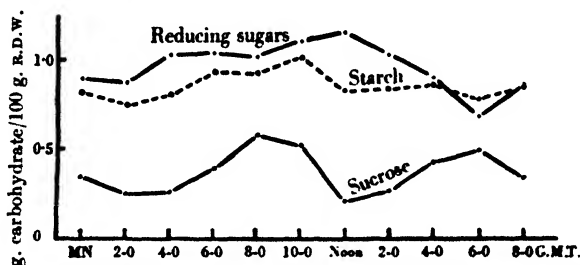
Fig. 1. Diurnal variations in carbohydrates of expanding leaves of healthy President at 20 days.

grounds. Priestley (1929), in a study of cell differentiation, has also stressed the high carbohydrate requirements of growing tissue in the formation of permanent structures, e.g. cell walls. It would seem, therefore, that in the young expanding potato leaf diurnal fluctuations in "free" carbohydrate would be reflexions of the disturbances in balance between utilization in support of growth and supply through carbon assimilation. Examination of the curves in Fig. 1 confirms this view. The accumulation of each form of carbohydrate with increasing light intensity between 4.0 a.m. and noon may be interpreted as the formation of sugars at a rate greater than that of utilization. From the order in which the curves begin their upward trend it would seem that reducing sugars accumulated first and that condensation to starch and sucrose was, subsequently, a concomitant of this accumulation. After noon, and with decreasing light intensity, the diminished values of reducing sugars may be taken to indicate a diminished rate of formation. When the concentration of reducing sugars reached a minimum value at 6.0 p.m. there is indication that reinforcement from sucrose and starch took place

and that these latter ultimately formed a source of supply of carbohydrate within the leaf.

Upon this interpretation, sucrose and starch serve as temporary storage products for excess carbohydrate formed in photosynthesis (cf. Clements, 1930; Barton Wright & McBain, 1932), and the balance of metabolism is controlled by the requirements of the leaf as a growing organ. Photosynthetic activities are conditioned to supply the materials of these requirements and, in consequence, the levels of sucrose and
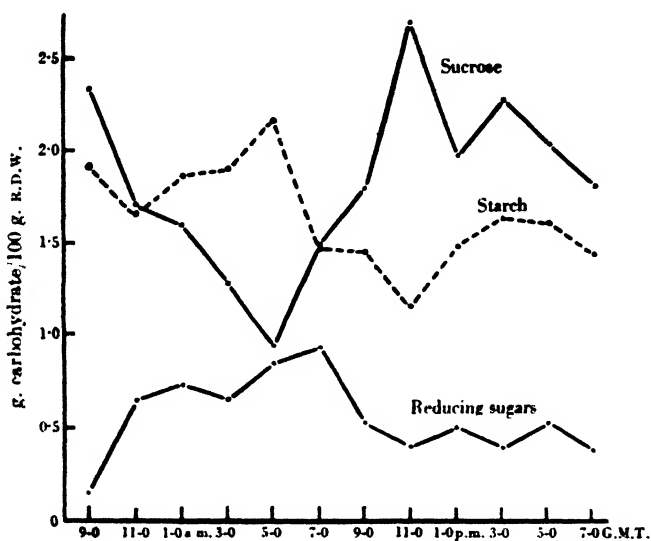
Fig. 2. Diurnal variations in carbohydrates of the leaves of healthy President at 44 days.

starch are low in relation to that of reducing sugars upon which, presumably, considerable demands are made in support of respiratory and synthetic activities. On the other hand, when conditions are favourable for the maintenance of carbon assimilation, that is during daylight, photosynthetic activity is sufficiently strong to throw the balance temporarily in favour of carbohydrate formation and the accumulation of reserve products.

President, 44 *days old*. The diurnal fluctuations and mean levels of carbohydrate in the leaves and petioles of 44 days old President (Fig. 2, Table II) are in close agreement with those previously recorded for potatoes in a vigorous vegetative condition prior to flowering (Davis & Sawyer, 1916; Clements, 1930; Barton Wright & McBain, 1932). The most

probable interpretation of these data is that carbohydrate formation during the day takes place in excess of utilization with the result that sucrose and starch accumulate as temporary reserve products. The progressively decreasing amounts of sucrose and starch during the night,

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Table II

Lamina

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Mean values	% R.D.W.	% total carbohydrate	Petiole % R.D.W.
Reducing sugars	0·564 ±0·061	14·42 ± 1·64	0·888
Sucrose	1·780 ±0·152	44·21 ±3·15	0·936
Starch	1·636 ±0·072	41·36 ±2·02	1·655

accompanied by increase in reducing sugars, points to the hydrolysis of starch to hexoses and the removal of soluble sugars in synthesis and/or by transport.

President, 86 days old. The carbohydrate curves for the leaves of President in a post-flowering and pre-senescent stage present a totally different appearance from those provided by the material sampled earlier in the season. It will be seen (Fig. 3) that between 9.0 a.m. and 1.0 p.m. the curves follow the normal daytime trend suggestive of photosynthetic activity resulting in carbohydrate formation in excess of utilization and
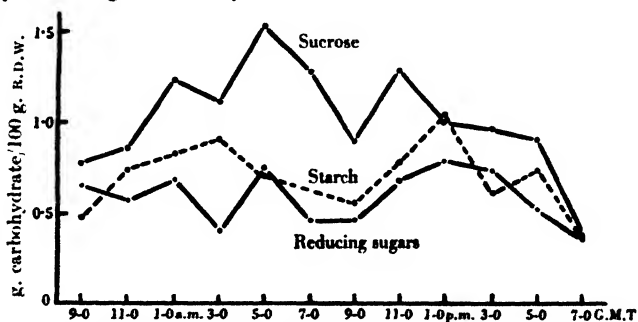
Fig. 3. Diurnal variations in carbohydrates of maturing leaves of healthy President at 86 days.

transport. The downward trend of all three curves between 1.0 p.m. and 7.0 p.m. is also normal and readily interpreted as showing a reduction of synthetic activity with decreasing light intensity and a consequential removal of the temporary storage products. Between 9.0 p.m. and 5.0 a.m., however, there was a marked increase in all forms of carbohydrate but chiefly in sucrose and starch.

Nocturnal increases in carbohydrate have been observed previously in leaves of potatoes approaching senescence (Clements, 1930; Barton

Wright & McBain, 1933 a) and also in mangold leaves sampled in September and October (Davis *et al.* 1916). It would appear, therefore, that the phenomenon is intimately associated with the onset of maturity and/or the transfer of carbohydrate reserves from leaves to winter storage organs. There is abundant evidence that annual plants at the end of their period of growth decrease considerably in dry weight (Blackman, 1919; Miller, 1931), and, although such a decrease cannot be substantiated in fact by the present data, the course of events shown (Fig. 3) would appear to indicate that during the pre-senescent period in the potato, carbohydrate reserves of the leaves are hydrolysed and removed to the storage organs. It would seem, indeed, that hydrolysis of the more permanent reserves, e.g. hemicelluloses, glucosides, and pentosans (Davis & Sawyer, 1916; Clements, 1930), may take place to such an extent during the night that there is a necessary recondensation of the simple sugars formed in hydrolysis to temporary storage products, in which form they may be held readily available for transport.

There are difficulties which arise in accepting this explanation of the nocturnal increase in carbohydrates, particularly with regard to the enhanced values of sucrose. The formation of sucrose is dependent upon supply of glucose and γ-fructose, and whilst there is strong direct evidence that glucose is the main product in the hydrolysis of polysaccharides and carbohydrate derivatives in general there is little proof that γ-fructose may arise in this way. Onslow (1931), however, has assembled evidence to show that starch and sucrose are interconvertible. The mechanism suggested is based upon the assumption that active, unstable γ-hexoses are formed in hydrolysis, and that in this active condition γ-glucose and γ-fructose are interconvertible. If this hypothesis be accepted and applied to the circumstances obtaining in the material under discussion, it would seem that the products of hydrolysis of reserve substances in the leaf are determined by two factors, first, the equilibria of the soluble sugars and, secondly, the rate of transport of these latter from the leaf. In other words, the conditions which determine the forms in which carbohydrate accumulates during the night when the primary forms of sugar are derived by hydrolysis are similar to those obtaining during the day when the primary sugars arise *de novo*. It has already been indicated that during the daylight period the normal synthesis of carbohydrate takes place, and it is suggested, therefore, that the hydrolysis of reserve products is controlled by the level of soluble carbohydrates, particularly of the unstable forms, within the leaf. Thus when photosynthetic activity is resumed in the early morning with the consequent

production of reducing sugars, the conditions become unfavourable for hydrolysis which, in consequence, ceases. At night, on the other hand, photosynthesis is no longer active and the conditions favour hydrolysis.

From the above discussion it would appear that metabolic activities in the maturing potato leaf are directed chiefly towards the supply of soluble sugars for transport and storage in the appropriate organs, namely, the tubers. A comparison to be made later of the petiolar data for each of the diurnal periods will corroborate this view.
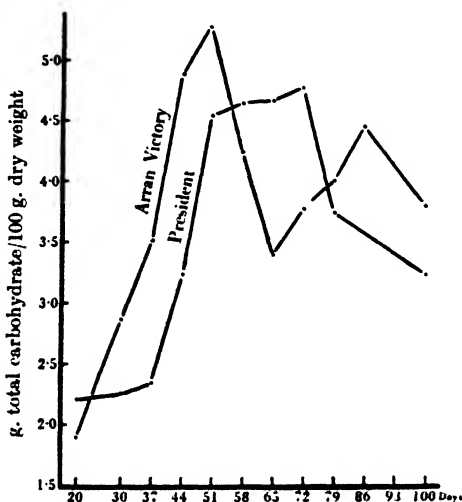
Fig. 4. Seasonal variations of total carbohydrate in healthy leaves of President and Arran Victory.

Seasonal series.

President. Seasonal variations in total weak-acid-hydrolysable carbohydrates of President leaves (Fig. 4) are expressive of three major periods of leaf metabolism. During the first period, 20–37 days, carbohydrate values remained at low levels although there was a slight tendency towards increase. Later this tendency became marked and the second period was characterized by a rapid increase in values culminating in a maximum between 51 and 72 days. Finally, there was a progressive diminution of carbohydrate from 72 days onwards. These data corroborate the interpretation placed upon the results of diurnal investigation, since they indicate that in expanding leaves utilization of carbohydrate is almost complete; that in fully expanded leaves carbohydrate is formed in

excess of utilization; and that in maturing leaves there is a withdrawal of carbohydrate reserves.

Arran Victory. The Arran Victory plants used in this investigation grew so strongly during the early part of the season that they rapidly became pot-bound and showed signs of premature ripening. In order to check this tendency each plant was repotted and supplied with a single teaspoonful of mixed fertilizer immediately after the seasonal samples were obtained on the 58th day. In response to this treatment a new period of growth was initiated. The effect of ripening and of treatment is seen plainly in the curve for total carbohydrates (Fig. 4).

The first four values on the curve were obtained from samples taken before the ripening effect was visible. During this period there was a rapid increase in carbohydrate content culminating in a maximum at 51 days. The premature ripening effect was apparently responsible for the rapid decrease in carbohydrate values between 51 and 58 days, but after treatment on the latter date, and presumably in response to the more favourable conditions, the decrease was checked at 65 days. Thereafter the curve shows an increase in carbohydrate to 86 days and a decrease from 86 days onwards. With the exception of the effect traceable to abnormal conditions of growth, the curve is very similar to that for President. This similarity is shown also in the seasonal distribution of the individual carbohydrates (Table III).

Table III

	$^o/_o$ R.D.W.			$^o/_o$ total carbohydrate		
Mean values	20 days	30 days	72 days	20 days	30 days	72 days
Reducing sugars	0·720	0·407	0·591	36·55	15·06	22·04
Sucrose	0·768	1·416	1·028	38·98	53·92	37·64
Starch	0·463	0·803	1·035	24·47	31·02	40·35

The major points of difference between the data for Arran Victory and for President are those of time and actual amounts of carbohydrate. Thus, Arran Victory appears to pass through its early stages of growth rapidly and, under normal conditions, might be expected to show maturity changes in metabolism earlier. In general, however, seasonal changes in the metabolism of carbohydrates in Arran Victory appear to follow the same lines as those adduced for President.

Petioles.

The estimates of carbohydrate variations in the petioles of both President and Arran Victory have been restricted to group analyses of

starch, sucrose and reducing sugars for each of the diurnal periods investigated. The mean diurnal values for each variety are given in Table IV and arranged in progressive series with age, irrespective of variety.

Table IV

	President 20 days	A. Victory 30 days	President 44 days	A. Victory 72 days	President 86 days
Mean values			% R.D.W.		
Starch	0·368	1·581	1·655	1·702	1·086
Reducing sugars	0·274	0·694	0·888	0·970	2·689
Sucrose	1·185	0·687	0·936	1·054	1·060
Soluble sugars	1·457	1·381	1·824	2·024	3·749

It will be seen from Table IV that soluble sugars in the petioles increased progressively throughout the season and reached very high values in the 86 days old material. Over the whole period, however, sucrose values remained almost constant, and the variations in soluble sugars were due entirely to increases in reducing sugars. These facts are in complete accord with those of other investigators, and the most probable interpretation of them is that they indicate a progressive increase in the amount of carbohydrate transported from the leaf. The increase is particularly well marked in the 86 days old plants and is corroborative, therefore, of the trend of metabolism suggested for these plants, namely, that depletion of carbohydrate in the leaf is directed towards transport of reserve materials from the leaf to the tubers.

The nature of the sugars concerned in translocation in the potato has given rise to two contrary views. Davis & Sawyer (1916) and Clements (1930) have expressed the opinion that since reducing sugars preponderate in the petioles they must be the chief sugars of transport. On the other hand, Barton Wright & McBain (1932) claim that sucrose is the translocatory sugar. Their data are conclusive in establishing an obligate connexion between sucrose and the phloem of the conducting organs and also in showing sucrose to be *a* form of sugar in which carbohydrate is translocated in the healthy potato plant. The data do not preclude, however, the possibility that reducing sugars are also concerned in transport. On the contrary, the results from the leaf-roll material would appear strongly to favour reducing sugars as the more readily translocated, since a positive gradient of hexoses between the midribs of the leaves and the lower stems was traced in these plants.

The present evidence, although inconclusive with regard to the function of sucrose in the petiole, supports the contention that reducing

sugars are the chief forms in which sugar is translocated, particularly towards the end of the plant's life, since reducing sugars in the petioles increased progressively in amount during the course of the season whilst sucrose remained almost constant. This latter fact may indicate that translocation of sucrose takes place at a maximum rate throughout the season and that it is restricted to certain channels, namely, the phloem. If this be the case, then it would seem that translocation of reducing sugars suffers no restrictions of this nature and has alternative channels as was suggested for the leaf-roll material of Barton Wright & McBain (1932).

IV. Variations in the Carbohydrate and Total Nitrogen Content of Mosaic Diseased Leaves as Compared with Normal Leaves

Before proceeding to a detailed comparison of the diurnal and seasonal variations in healthy and mosaic-infected plants, it is convenient to draw attention to a salient feature of the results to be presented. It will be observed from the data for each series of comparisons that the *gross* levels of the carbohydrates with regard to seasonal changes in level, and the *gross* trend of diurnal and seasonal variation are closely similar in both healthy and diseased material. These facts of similarity may be used in dual fashion: first, to double the strength of the evidence relative to metabolism in the healthy plant; and secondly, to establish the principle that the *gross* metabolism of the diseased plant is essentially that of the healthy plant. An acceptance of this principle at this stage will avoid constant repetition in drawing attention to the fact that the differences to be found in detailed comparison are small in relation to the similarities in wide comparison.

Experimental observations

Diurnal series.

President, 20 days old. The slight tendencies towards difference between the curves and mean values for mosaic-diseased and healthy leaves of 20 days old President (Fig. 5, Table V) are worthy of examination in view of their bearing on the further results. The curves show that reducing sugars in the diseased laminae tended to fluctuate at a lower mean level during the day and at a higher mean level during the night. The diurnal mean (Table V), however, was slightly lower than that for the healthy laminae. Sucrose values, on the other hand, were almost identical for both series of plants at all times of day and also in the diurnal means.

Starch fluctuations resembled those of reducing sugars, in that they took place about a lower mean value in the diseased laminae, and it will be observed that they showed a tendency to lag behind corresponding fluctuations in the healthy laminae. When considered in proportionate relationships, the mean values for reducing sugars were almost identical
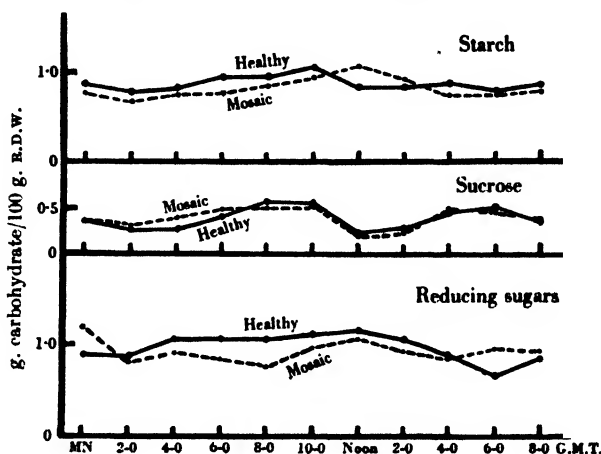
Fig. 5. Diurnal variations in carbohydrates of expanding leaves of healthy and mosaic President at 20 days.

Table V

Mean values	Healthy	Mosaic	Difference	D/E_D
% B.D.W.:				
Reducing sugars	0.973 ± 0.040	0.939 ± 0.036	0.034 ± 0.054	0·63
Sucrose	0.376 ± 0.035	0.382 ± 0.034	0.006 ± 0.049	0·12
Starch	0.854 ± 0.022	0.803 ± 0.034	0.051 ± 0.041	1·24
% Total:				
Reducing sugars	44.20 ± 1.46	44.24 ± 1.23	0.04 ± 1.91	0·02
Sucrose	16.95 ± 1.36	17.99 ± 1.47	1.04 ± 2.01	0·52
Starch	38.91 ± 0.36	37.77 ± 1.16	1.14 ± 1.21	0·94

in both series of plants, and the balance of difference was held by slightly higher sucrose and slightly lower starch in the diseased leaves.

The main direction of metabolism in the leaves of normal potatoes at this stage of development has been postulated to be that of carbohydrate formation and utilization in tissue synthesis. The facts for the diseased laminae give support to this hypothesis and, therefore, the slight differences which have been noted must be ascribed to either an excess of utilization or a diminished rate of formation of carbohydrate in these

laminae. The fact that reducing sugars and starch were most markedly lower during the earlier part of the photosynthetic period is suggestive of a lower rate of formation as most probable. Since the higher values of reducing sugars in the diseased laminae during the night may be taken to indicate that the demand made upon these sugars in support of respiration and synthesis was not as great as in the healthy laminae, then the argument in favour of a lower rate of carbohydrate formation is considerably strengthened. The lag in starch fluctuations together with lower starch values on both actual and proportional bases lead to the further suggestion that starch formation is subject to additional impediment in the diseased laminae.

Table VI

Mean values	Healthy	Mosaic	Difference	D/E_D
% R.D.W.:				
Reducing sugars	0.564 ± 0.061	0.464 ± 0.066	0.100 ± 0.090	1.11
Sucrose	1.780 ± 0.152	1.601 ± 0.166	0.179 ± 0.225	0.80
Starch	1.636 ± 0.072	1.434 ± 0.081	0.202 ± 0.108	1.87
% Total:				
Reducing sugars	14.42 ± 1.64	13.63 ± 2.05	0.79 ± 2.63	0.30
Sucrose	44.21 ± 3.15	44.71 ± 3.11	0.50 ± 4.43	0.11
Starch	41.36 ± 2.02	41.66 ± 2.78	0.30 ± 3.44	0.10

President, 44 days old. If the effect of virus infection is to decrease the efficiency of carbohydrate production, as indicated by the data for the young developing leaves, then stronger evidence of this diminished efficiency should be obtained when the trend of carbohydrate metabolism in the leaf is directed towards the formation of storage carbohydrates. The comparative data in Table VI show that in 44 days old leaves all three forms of carbohydrate were lower in the diseased material. The suggestion thus receives support from this fact alone. The general reduction in carbohydrate, however, was not the only difference between the two series, as will be seen by comparing the curves for reducing sugars (Fig. 6). These curves show that reducing sugars in the diseased laminae tended towards greater values during the photosynthetic period, 7.0 a.m. to 7.0 p.m., and towards lower values during the night. The differences in fluctuations of reducing sugars are the reverse of those found in the first diurnal series, and their interpretation must be sought in the light of the differences in metabolism between the leaves sampled at 20 and at 44 days. In the former series the products of photosynthesis were found to be directed mainly towards utilization in tissue synthesis. Reducing sugars, i.e. the basic carbohydrates of this synthesis, pre-

dominated in the leaf. Disturbances in metabolism were reflected directly in the fluctuations of reducing sugars, and the fact that these latter were of smaller values during the day and of greater values at night in the diseased leaves has been taken to indicate that they were formed to a less extent in photosynthesis and utilized to a less extent in general synthesis. In the 44 days old material, however, the picture of metabolism drawn from the carbohydrate relations of the healthy leaf was that of
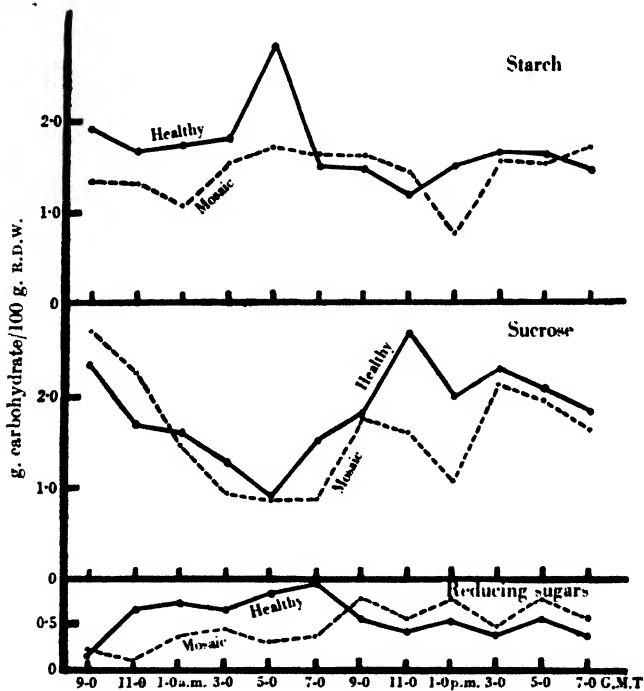
Fig. 6. Diurnal variations in carbohydrates of the leaves of healthy and mosaic President at 44 days.

carbohydrate synthesis directed to the laying down of both temporary and permanent carbohydrate reserves. Sucrose and starch were shown to serve as temporary storage products and to predominate largely over reducing sugars. Consequently, disturbances in metabolism should be most readily observed in the formation of sucrose and starch, i.e. in the main direction of synthesis. The enhanced values of reducing sugars in the diseased leaves during the photosynthetic period is not interpreted as indicative of an increased rate of carbon assimilation, therefore, but of a

reduced rate of formation of sucrose and starch leading to lower values of these latter. Conversely, the lower values of reducing sugars during the night may be used as evidence that the demand for these sugars was greater than the supply made available by hydrolysis of the temporary reserves and hence that the lower values were brought about by an impeded rate of hydrolysis of starch and/or sucrose. Since the starch fluctuations in the diseased laminae tended to lag behind those in the healthy, it would seem that the disturbed relationships were primarily those between reducing sugars and starch.
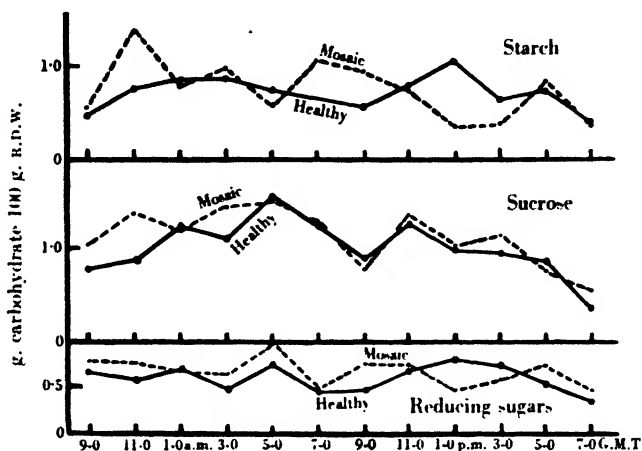
Fig. 7. Diurnal variations in carbohydrates of maturing leaves of healthy and mosaic President at 86 days.

President, 86 days old. The comparative curves for the third diurnal series (Fig. 7) show that reducing sugars and sucrose fluctuated in similar fashion in both healthy and diseased laminae and that starch fluctuated inconsistently with time in the diseased laminae. From the comparison of mean levels (Table VII) it is evident that with approaching senescence the relations of the carbohydrates in the diseased leaf, as compared with those in the healthy leaf, were different from those observed earlier in the season. Reducing sugars and sucrose were of higher values in the diseased laminae, whilst starch values, although lower, were appreciably near those of the healthy leaf. There was, therefore, a tendency towards a relative accumulation of all three carbohydrates in the diseased leaves. This is in complete agreement with previous observations on the maturing leaves of mosaic diseased potatoes (Barton Wright & McBain, 1933*a*; Stone, 1936).

It has already been suggested that the metabolism of the healthy leaf at this stage of development is directed chiefly towards the breakdown of reserve carbohydrates and transport of soluble sugars to the tubers. In these circumstances, the accumulation of soluble sugars and starch in the diseased laminae may be due to an accelerated rate in the hydrolysis of reserve carbohydrates and/or a retarded rate of transport. The fact that reducing sugars in the petioles of the diseased material were significantly lower than in the healthy (Table VII) suggests that there was a marked interference with translocation. This again is in agreement with the findings of Barton Wright & McBain (1933a).

Table VII

Mean values	Healthy	Mosaic	Difference	D/E_D
		Lamina		
% R.D.W.:				
Reducing sugars	0.594 ± 0.039	0.666 ± 0.046	0.072 ± 0.060	1.20
Sucrose	1.011 ± 0.082	1.120 ± 0.083	0.109 ± 0.117	0.93
Starch	0.801 ± 0.107	0.739 ± 0.092	0.062 ± 0.141	0.44
% Total:				
Reducing sugars	25.55 ± 1.67	26.94 ± 1.44	1.39 ± 2.21	0.63
Sucrose	41.82 ± 1.48	44.40 ± 2.25	2.58 ± 2.70	0.96
Starch	32.54 ± 2.11	28.42 ± 2.34	4.12 ± 3.15	1.31
		Petiole		
% R.D.W.:				
Reducing sugars	2.689 ± 0.234	1.505 ± 0.112	1.184 ± 0.259	4.57
Sucrose	1.060 ± 0.270	0.946 ± 0.092	0.114 ± 0.285	0.40
Starch	1.086 ± 0.102	0.909 ± 0.041	0.177 ± 0.110	1.61

Seasonal series.

As in the case of the diurnal data, similarity is the most striking feature of the curves showing seasonal variations in the total carbohydrates of the healthy and diseased leaves. There are slight differences between the comparable curves, however, and an examination of these will serve to strengthen the various suggestions arising out of the differences observed in diurnal metabolism.

An inspection of the curves for President (Fig. 8) reveals a tendency towards higher carbohydrate values in the diseased leaves between 20 and 37 days but towards lower values during the greater part of the period of carbohydrate accumulation.

It has been suggested that in the developing leaves of the healthy controls there was a balance between carbon assimilation and carbohydrate utilization in support of growth. Later, when the leaves were fully expanded, the balance was destroyed and carbohydrate formation exceeded its utilization in growth with the result that storage products

were accumulated in increasing quantities. The higher values of total
carbohydrate in the diseased leaves at the beginning of the period of
accumulation may be taken to indicate, therefore, that the diversion of
assimilated carbon from utilization in growth to the laying down of
storage products took place earlier in these leaves than in the healthy
leaves. The curves show that the carbohydrate content of the latter
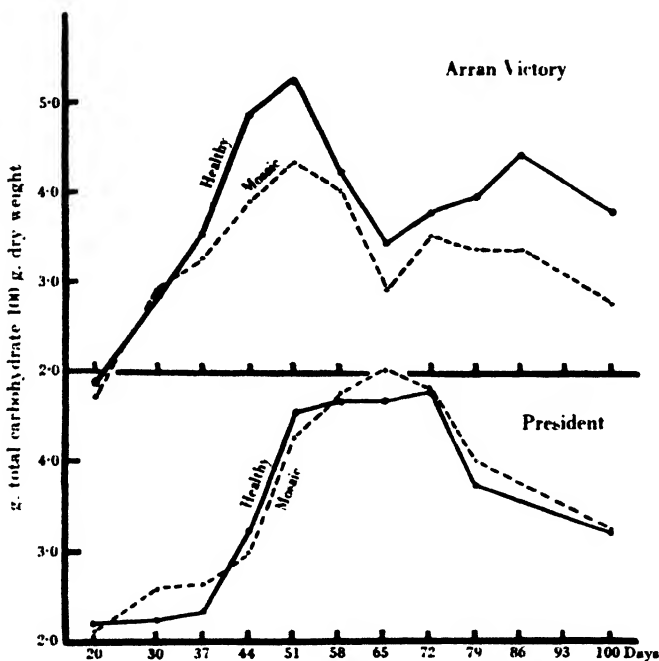
Fig. 8. Seasonal variations in some carbohydrates of the leaves of healthy
and mosaic President and Arran Victory.

increased at a greater rate than that of diseased leaves with the result
that carbohydrate values were at a higher level in the healthy leaves. This
points to a reduced efficiency in carbon assimilation in the diseased
leaves and leads to the suggestion that the earlier reversal was caused by
a retardation in growth activities within these leaves resulting in a
lessened demand for carbohydrate in the support of tissue synthesis.

Increase in the carbohydrate content of the healthy leaves was rapid
at first but diminished in course of time with the establishment of a
fairly steady maximum at 51–72 days. The carbohydrate content of the

diseased leaves, however, continued to increase after that of the controls had reached its maximum with the result that from 58 days onwards there was a greater amount of carbohydrate in the diseased leaves than in the healthy. There is no reason for assuming that increased photosynthetic activities of the diseased leaves were responsible for the higher carbohydrate content. On the other ·hand, there is justification for adopting the alternative assumption that diminished utilization of carbohydrate in the diseased plants affected the comparative levels between the two series. It will be remembered that the diurnal series of samples taken at 44 days and 86 days were obtained from plants in their pre-flowering and post-flowering stages, respectively, and it follows that the interval between these samples covered the flowering period of the material under review. There is evidence that infection of President with potato virus X is accompanied by a reduction of the number of flower trusses and also by a reduction of the number of flowers formed per truss (unpublished data). Consequently, it may be expected that there would be a lessened demand upon carbohydrate for the maintenance of growth and development of flowers in diseased plants as compared with healthy plants. The data for 86 days old President have been interpreted as showing that there were disturbances affecting translocation which resulted in relatively higher carbohydrate contents in the diseased plants. Impeded translocation, therefore, may also have been a determining factor in the accumulation of carbohydrate in the diseased plants from 58 days onwards. The two factors, impeded transport and reduced flowering capacity, may be causally linked, and the observed effects on carbohydrate economy in diseased plants may be due to either or both. Neither affect sugar formation, however, but both affect its utilization outside the leaf and, therefore, there are grounds for support of the assumption that the higher levels of carbohydrate in diseased leaves during the flowering period are due to diminished demands upon carbohydrate for use outside the leaves.

The higher values of carbohydrate in the diseased leaves during the whole period of carbohydrate decrease with approaching maturity give support to the observations recorded for 86 days old President but throw no further light on the cause of disturbance.

If the seasonal curves for Arran Victory are now examined it will be seen that after a transient reversal in carbohydrate values at 30 days, carbohydrate in the diseased leaves remained at a lower level throughout the season. The trend of fluctuation was the same in both healthy and diseased leaves, however, and the response to manurial treatment was

apparently greater and was maintained over a longer period in the healthy leaves than in the diseased leaves. In consequence the differences in carbohydrate contents of the two series widened considerably towards the end of the season.

Total nitrogen.

The diurnal samples of President taken at 44 and 86 days, the diurnal samples of Arran Victory taken at 30 and 72 days, and each seasonal sample of both varieties were analysed for total nitrogen. In every comparison the diseased material was found to have a higher nitrogen content and the differences of the mean diurnal values (Table VIII) were highly significant. The data in Table VIII indicate the main features of

Table VIII

| Variety | Age in days | Total nitrogen as g. 100 g. R.D.W. | | | |
		Healthy	Mosaic	Difference	$D E_D$
Arran Victory	30	6·829 ± 0·060	7·090 ± 0·022	0·261 ± 0·064	4·08
President	44	6·555 ± 0·047	6·875 ± 0·051	0·320 ± 0·069	4·64
Arran Victory	72	5·546 ± 0·056	6·138 ± 0·069	0·592 ± 0·107	5·53
President	86	5·435 ± 0·037	5·867 ± 0·040	0·432 ± 9·055	7·85

the investigation, namely, that the amount of nitrogen decreased with increasing age of the plant; that nitrogen was greater in the diseased series; and that the differences widened with increasing age of plant. No comparative curves are given, since in every case they were almost parallel and throw no light on the causes of disturbance in nitrogen metabolism.

V. DISCUSSION

It has been emphasized that the comparison between carbohydrate variations, over diurnal and seasonal periods, has established a similarity in the gross metabolism of carbohydrates in normal and mosaic-infected potato leaves. Nevertheless, a detailed examination of the data has provided evidence that modifications of the fundamental metabolism, slight though definite in character, may arise in consequence of mosaic infection. At each stage of diurnal investigation there were lower starch values in the diseased laminae, and slight but noticeable differences in carbohydrate variations pointed consistently towards impediment in starch formation and hydrolysis. In addition, there was indication of an interference with the utilization of sugars during the early stages of growth when the leaves were actively engaged in tissue synthesis. This interference became pronounced later, and it was suggested that the

cause was to be found in a premature retardation of growth activities which was reflected in the diversion of the products of photosynthesis from utilization in tissue synthesis to the formation of storage carbohydrates. This change in metabolism proceeded in normal fashion and merely anticipated a similar change in the healthy leaf. The difference in trend of metabolism between the two series was, moreover, transient, and subsequently the only differences to be found were in slightly reduced amounts of carbohydrate and in the persistent interference with starch elaboration in the diseased leaves. A further apparent disturbance was noted during the flowering period of growth in President, but again, though the evidence was indirect, the cause was sought not in carbohydrate metabolism *per se* but in its relations with the general metabolism of flower production.

During the pre-senescent stages of growth an interference with translocation became evident. In consequence there was a slight disorganization of metabolism due to the accumulation of soluble sugars, but no trace was found of any large interference with the normal processes of carbohydrate degradation characteristic of the period.

The causation of these slight disturbances in the carbohydrate economy of mosaic-infected potato leaves may be sought in two possible sources. In the first place, the slightly reduced efficiency in carbon assimilation, the disturbed starch-sugar relationships, and the impeded translocation, may be traced directly to the pathological changes brought about by the virus. Smith (1924) and Clinch (1931) have made anatomical studies of potato leaves affected with mosaic diseases, and their observations show that histopathological symptoms within leaves affected with mild or simple mosaic are, in general, restricted to the mottled areas. In these areas the mesophyll is undeveloped, there is a reduction in size and number of chloroplasts, and the chlorophyll has a paler appearance. Clinch has shown further that the extent of these modifications is proportional to the external visible symptoms of the disease and that there are marked abnormalities in the starch content of cells in the chlorotic areas. In contrast with the evidence of the above authors, Rochlin (1930) has stated that pathological symptoms consequent upon infection with mosaic viruses are not restricted to the mottled areas but may spread to destructive changes in the conducting tissues. These conclusions are qualified, however, by the observation that the extent of internal symptoms is correlated with the severity of the external symptoms.

Since the disease symptoms upon the leaves of the two potato varieties used in the present investigation were confined to small, slightly

chlorotic, mottled areas, and in view of the anatomical evidence the slight disturbances which have been observed in diurnal carbohydrate metabolism would appear to be directly associated with the presence of the mottled areas. It would seem that the greater portion of the leaf surface functions normally with regard to the formation and utilization of sugars, resulting in a gross similarity between observations for normal and diseased leaves, but that in the relatively small areas in which pathological symptoms are manifested the formation of sugars in photosynthesis does not take place, or takes place to a small extent only, and the formation and degradation of starch are subject to impediment. This conclusion is in agreement with that of Cook (1926) who has shown that in sugar cane the green areas of mosaic-infected plants synthesize sugars in the normal manner but that the products of photosynthesis, particularly starch, are reduced in proportion to the amount of visible infection.

The interference with translocation towards the end of the season may, in the light of Rochlin's evidence (1930), be ascribed to pathological effects, but in the absence of specific data upon the channels through which reducing sugars are removed from the leaves, no great stress can be laid upon this suggestion.

A second cause of modification in the carbohydrate metabolism of mosaic-infected leaves has been traced to disturbances affecting primarily the growth activities of the plant. Thus, in the early stages of development, indication was obtained that growth was retarded in the diseased leaf earlier than in the healthy leaf. During the flowering stage, also, there was indication that carbohydrate was not used in support of flower production to the same extent as in the healthy plant. There has been no evidence to suggest, however, that disturbances in carbohydrate metabolism were the causes of these interferences with growth. It would seem that they were effects and that the causes must be sought in some other aspect of general metabolism.

The obligate parasitism of viruses suggests that their auto-synthesis is dependent upon the partly synthesized intermediate products of metabolism and upon the physiological conditions extant in living tissue. More particularly, in view of the protein nature of mosaic viruses (Stanley, 1935), it would seem that the metabolism of nitrogen would provide a suitable source of materials for this synthesis. On this hypothesis the pathological symptoms and retarded growth activities which give rise to disturbances in carbohydrate metabolism may well be direct manifestations of a disorganized nitrogen metabolism.

VI. Summary

The effects of potato virus X upon the carbohydrate and total nitrogen contents of potato leaves have been followed over diurnal and seasonal periods in the varieties President and Arran Victory.

The gross metabolism of carbohydrates has been found to be similar in both mosaic-diseased and normal leaves.

Three major trends of carbohydrate metabolism, each of which may be identified with a particular stage of leaf development, have been recognized and discussed.

Slight modifications of the fundamental metabolism of diseased leaves have been found in a reduced photosynthetic activity, disturbed starch-sugar relationships, and an impediment in translocation during the pre-senescent stage. All these have been ascribed to secondary effects arising out of the pathological change brought about as a result of virus infection.

Transient modifications in carbohydrate metabolism have been traced to prematurely retarded growth activities leading to a diminished demand for carbohydrate in support of growth in the diseased plants.

A significantly greater content of nitrogen has been found in diseased leaves at all stages of growth, and it has been suggested that the pathological symptoms and prematurely retarded growth activities which give rise to disturbances in carbohydrate metabolism are direct manifestations of a disorganized nitrogen metabolism.

REFERENCES

BARTON WRIGHT, E. & MCBAIN, A. (1932). Studies in the physiology of the virus diseases of the potato. *Trans. roy. Soc. Edinb.* 57, 309-49.

—— —— (1933a). Studies in the physiology of the virus diseases of the potato. II. *Ann. appl. Biol.* 20, 525-48.

—— —— (1933b). Studies in the physiology of the virus diseases of the potato. III. *Ann. appl. Biol.* 20, 549-89.

BLACKMAN, V. H. (1919). The compound interest law and plant growth. *Ann. Bot., Lond.*, 33, 353-60.

BUNZEL, H. H. (1914). Oxidases in healthy and in curly dwarf potatoes. *J. agric. Res.* 2, 373-404.

CLEMENTS, H. F. (1930). Hourly variations in carbohydrate content of leaves and petioles. *Bot. Gaz.* 89, 241.

CLINCH, PHYLLIS (1931). Cytological studies of potato plants affected with certain virus diseases. *Sci. Proc. roy. Dubl. Soc.* 20 (N.S.), 143-72.

COOK, M. T. (1926). Photosynthesis of the sugar cane mosaic plant. *J. Dep. Agric. Porto Rico*, 10, 239-42.

DAVIS, W. A., DAISH, A. J. & SAWYER, G. C. (1916). Studies of the formation and translocation of carbohydrates in plants. I. *J. agric. Sci.* 7. 255-326.

DAVIS, W. A. & SAWYER, G. C. (1916). Studies of the formation and translocation of carbohydrates in plants. III. *J. agric. Sci.* 7, 352–84.

FISHER, R. A. (1932). *Statistical Methods for Research Workers.* London.

LINK, K. P. & TOTTINGHAM, W. E. (1923). *J. Amer. chem. Soc.* 45, 439.

MASON, T. G. & MASKELL, E. J. (1928). Studies on the transport of carbohydrates in the cotton plant. I. *Ann. Bot., Lond.,* 42, 1–65.

MILLER, E. C. (1931). *Plant Physiology.* New York and London.

ONSLOW, M. W. (1931). *The Principles of Plant Biochemistry.* Cambridge.

PEARSALL, W. H. & WRIGHT, A. (1929). The proportions of soluble and insoluble nitrogenous materials in fresh and dried plant tissue. *Proc. Leeds phil. lit. Soc.* 2, 27–31.

PRIESTLEY, J. H. (1929). Cell growth and cell division in the shoot of the flowering plant. *New Phytol.* 28, 54–81.

RANKER, E. R. (1925). Determination of total nitrogen in plants and plant solutions: a comparison of methods with modifications. *Ann. Mo. bot. Gdn,* 12, 367–80.

ROCHLIN, E. (1930). Zur Anatomie der mosaikkranken Kartoffelpflanzen. *Phytopath. Z.* 2, 455–68.

SCHAFFER, E. A. & HARTMANN, A. F. (1921). The idiometric determination of copper and its uses in sugar analysis. *J. biol. Chem.* 45, 365.

SMITH, K. M. (1924). On a curious effect of mosaic disease upon the cells of the potato leaf. *Ann. Bot., Lond.,* 38, 150.

STANLEY, W. M. (1935). Isolation of a crystalline protein possessing the properties of tobacco mosaic virus. *Science,* N.S. 81, 644–5.

STONE, W. E. (1933). Normal growth of potato leaves in greenhouse and field. *J. agric. Res.* 46, 565–78.

⸺ (1936). Growth, chemical composition and efficiency of normal and mosaic potato plants in the field. *J. agric. Res.* 52, 295–309.

WENT, F. A. F. C. (1898). Chemisch-physiologische Untersuchungen über das Zuckerrohr. *Jb. wiss. Bot.* 31, 289–344.

OBSERVATIONS ON APPLE CANKER

I. THE DISCHARGE AND GERMINATION OF SPORES OF *NECTRIA GALLIGENA* BRES.[1]

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(With Plate XXIII and 4 Text-figures)

CONTENTS

INTRODUCTORY

THE term apple canker is loosely used to include the lesions resulting from attacks of a number of fungi (Ogilvie, 1935), or from severe woolly aphis attack, or from certain physiological disorders producing similar symptoms. It is applied to almost any kind of open wound surrounded by more or less necrotic tissue. The observations described in this paper have been confined to the canker disease due to *Nectria galligena* Bres.,

[1] Read before the Association on 9 December 1938.

termed in America European canker to distinguish it from similar cankers having their origin in the attacks of other parasitic fungi.

The disease is common in the European countries and is now established in the north-western and north-eastern fruit-growing areas of U.S.A. and in eastern Canada (Dickson, 1925; Zeller, 1926). In addition to causing cankers which may in time girdle and kill a branch, the fungus also causes a calyx- or "eye"-rot of apples and pears (Dillon Weston, 1927; Osterwalder, 1931).

In the study of any disease, one of the chief factors to be considered is the prevalence of infective material. The purpose of this investigation has therefore been to examine the extent to which spores of the fungus are present under natural conditions throughout the year. Two spore stages of the fungus are recognized as responsible for the spread of the disease, viz. macroconidia and ascospores. The macroconidia are produced in very large numbers in small, dirty white sporodochia which push through the killed bark of the host, not infrequently in roughly concentric circles round the point of infection. It is usually this stage which first becomes apparent on the lesions appearing in spring and summer on one- and two-year-old wood, often ringing these young growths.

The macroconidia are followed later in the season by the winter stage, appearing as irregular groups of red perithecia, from the apical pores of which large numbers of ascospores are liberated. The perithecia are similar in colour to the eggs of red spider, but are about twice the diameter of the latter and differ also in that they are confined to cankered bark.

THE CAUSAL ORGANISM

Historical

European canker is caused by a fungus of the genus *Nectria*, though agreement was not reached for some time concerning the species responsible. Historical reviews of the mycological literature on the subject are given by Cayley (1921) and Zeller (1926). Zeller concludes that the earlier work deals with two or possibly three separate organisms.

In 1865 Tulasne described *N. ditissima* from beech, and this organism was subsequently shown to be the cause of canker in beech (Hartig, 1880) and in apple (Goethe, 1904). The conidial stage of the fungus, first described by Willkomm in 1866, also from beech, as *Fusidium candidum*, was transferred by Lindau (1909) to *Fusarium Willkommii*.

Nectria ditissima Tul. was accepted by von Höhnel & Weese (1910) as synonymous with *N. coccinea* (Pers.) Fr. Weese (1911), working with *N.*

ditissima Tul., *N. coccinea* (Pers.) 'Fr., and *N. galligena* Bres. (Bresadola, 1901), came to the conclusion that canker in fruit trees and in timber trees is due to *N. galligena* Bres., which he distinguished from *N. ditissima* Tul. by differences in the structure of the perithecium, in the characters of the spores, and chiefly as a canker producer. *N. galligena* Bres. he found associated with definite cankers, whereas the other two species were confined to smooth areas of bark. Weese confirmed the statement of Appel & Wollenweber (1910) that *Fusarium Willkommii* Lindau was the conidial stage of *Nectria galligena* Bres. Wollenweber (1913*a*) also attributes canker in apple trees to *N. galligena* Bres. (= *Fusarium Willkommii* Lindau).

Wollenweber (1913*b*) segregated those forms having macroconidia with rounded ends from the genus *Fusarium* Link as a distinct genus *Cylindrocarpon* Wr., and the conidial stage of *Nectria galligena* Bres. was transferred from *Fusarium* (= *Cylindrocarpon*) *Willkommii* to *Cylindrocarpon mali* (Allesch.) Wr. = *Fusarium mali* Allesch. (1892). The name *Cylindrocarpon Willkommii* (Lind.) Wr. was given to the conidial stage of *Nectria ditissima* Tul. This ascription has generally been accepted by later workers, who include Cayley (1921) in this country, Zeller (1926) in Oregon, U.S.A., and Richter (1929) on the continent.

Other important papers in recent years add further points. Westerdijk & van Luijk (1924), from investigation of the fungi in pure culture, distinguish between *N. galligena* Bres. and *N. coccinea* (Pers.) Fr. on the basis of differences in ascospore dimensions--17-18 and 12-13μ in length respectively. They are of the opinion that if the biometric method of ascospore measurement were adopted, *N. ditissima* Tul. would fall into one of these two species. The characters of the perithecial wall and of the macroconidia they find too variable to serve as reliable criteria for classification.

Richter (1929) disagrees and claims that the conidial characters are of importance in distinguishing between *N. galligena* Bres. and *N. ditissima* Tul. By plotting the frequency curves for ascospore length, a sharp difference is found between the steep curves for four strains of *N. coccinea* (Pers.) Fr. and the much flatter curves for two strains of *N. galligena* Bres. The curves for *N. ditissima* Tul. and *N. punicea* (K. & Schm.) Fr. are intermediate in form. These two species are separated by differences in the ratio of length/breadth of the ascospores. Conidial characters also differ in the four species. The conidia of *N. ditissima* Tul. are longer and more slender (4·9μ) than those of *N. galligena* Bres. (5·6μ), which, like those of *N. punicea*, are also more curved. Inoculation experiments

showed that *N. galligena* would readily produce typical cankers on apple, less readily on pear and beech, while *N. ditissima* gave cankers on all three. *N. coccinea* and *N. punicea* proved non-pathogenic in Richter's experiments.

Moritz (1930) reports inoculation experiments with twenty-two strains of *Nectria* spp. His results emphasize the existence of strains of *N. galligena* Bres. differing in virulence, some being able to produce cankers on a range of host trees, including apple, while others were entirely non-pathogenic. Moritz differs from Richter in finding that *N. coccinea* (Pers.) Fr. is capable of causing canker of apple shoots, and finds no evidence of host specialization.

Wiltshire (1921), in an account of the method of infection by the Apple Canker fungus through leaf scars, described it under the name of *N. ditissima* Tul. though acknowledging that doubt existed as to the correct nomenclature. Cayley (1921), from a study of the fungus, chiefly in pure culture, considered that the organism causing canker on Apple in England was *N. galligena* Bres. No comprehensive measurements of the spores of the fungus are given, however, to provide a comparison with the descriptions of Wollenweber (1913*b*, 1929) and Richter (1929).

Descriptive

(1) *Macroconidia.*

In the present investigation, conidia from pure cultures of different strains of the fungus on various media have been measured. The media used have been malt-extract agar, oatmeal agar, and wheatmeal agar; while, for comparison, conidia have also been measured from cankered bark kept in moderately damp conditions in the laboratory. All spores have been measured in distilled water, care being taken to discontinue measurement before the result was affected by the onset of germination, which, at laboratory temperatures, may be perceived after about 3 hr. The media used had little influence on the dimensions of the macroconidia of similar septation. Malt-extract agar and oatmeal agar tended to give conidia of fewer septa than those developing on wheatmeal agar, which very closely resembled the conidia developing in the laboratory on naturally cankered bark.

The mean results of the measurements of macroconidia of three strains of the fungus are assembled in Table 1. The strains were isolated as follows:

Strain 1. From a canker on apple resulting from natural infection of a pruning cut. Monoconidial isolation.

Strain 2. From a large superficial canker apparently of recent development and almost girdling a large branch of apple.

Strain 3. From a leaf scar infection of apple. Monoconidial isolation.

Table I. *Comparison of spore measurements (in* μ)

Nectria galligena Bres.

Macroconidia. *Cylindrocarpon mali* (Allesch.) Wr.

Strain	1-septate	2-septate	3-septate	4-septate	5-septate	6-septate	7-septate
Strain 1:							
Wheatmeal agar	—	—	$35 \times 5 \cdot 0$	$43 \times 5 \cdot 4$	$61 \times 5 \cdot 6$	—	—
Oatmeal agar	$25 \times 4 \cdot 0$	—	$40 \times 5 \cdot 0$	$51 \times 5 \cdot 3$	$59 \times 5 \cdot 6$	—	—
Cankered bark	$22 \times 4 \cdot 0$	$28 \times 4 \cdot 4$	$38 \times 4 \cdot 8$	$45 \times 5 \cdot 0$	$54 \times 5 \cdot 4$	$60 \times 5 \cdot 6$	—
Strain 2:							
Wheatmeal agar	$22 \times 3 \cdot 9$	—	—	$45 \times 5 \cdot 3$	$61 \times 5 \cdot 6$	$69 \times 5 \cdot 9$	$71 \times 6 \cdot 0$
Malt-extract agar	—	—	$39 \times 5 \cdot 2$	$50 \times 5 \cdot 4$	$52 \times 5 \cdot 4$	—	—
Strain 3:							
Wheatmeal agar	—	—	—	—	$56 \times 5 \cdot 4$	—	—
Malt-extract agar	$24 \times 4 \cdot 5$	$29 \times 4 \cdot 8$	$39 \times 5 \cdot 3$	$48 \times 5 \cdot 4$	—	—	—
Authority							
Wollenweber, 1913	—	—	—	$48\text{–}50 \times 4 \cdot 8\text{–}5 \cdot 8$	$54\text{–}62 \times 5\text{–}6$	68×6	68×6
1929	—	—	$41 \times 5 \cdot 0$	—	$56 \times 5 \cdot 5$	...	—
Richter, 1929	—	—	$38 \times 5 \cdot 0$	$47 \times 5 \cdot 2$	$60 \times 5 \cdot 5$	$68 \times 5 \cdot 8$	$76 \times 5 \cdot 75$

Nectria ditissima Tul.

Macroconidia. *Cylindrocarpon Willkommii* (Lind.) Wr.

Authority							
Wollenweber, 1929	—	—	$37 \times 3 \cdot 5$	—	$72 \times 4 \cdot 4$	-	$89 \times 4 \cdot 4$
Richter, 1929	—	—	—	—	$69 \times 4 \cdot 9$	$77 \times 4 \cdot 9$	$85 \times 4 \cdot 8$

The measurements given for *Cylindrocarpon mali* (Allesch.) Wr. by Wollenweber (1929) and Richter are included for purposes of comparison, as also are those for *C. Willkommii* (Lind.) Wr., the conidial stage of *Nectria ditissima* Tul. The conclusion is drawn that the conidial stage of the fungus isolated at Long Ashton is the same organism as that described by Wollenweber (1913*b*, 1929) and by Richter (1929) as *Cylindrocarpon mali* (Allesch.) Wr.

It was sometimes observed that a strain of the fungus, after about six months of pure culture, ceased to produce typical macroconidia. The spores then produced were short and possessed few or no septa. Typical macroconidia, on the other hand, have been formed in large numbers in strains of the fungus after culture for nearly two years.

(2) *Ascospores.*

Perithecia were produced in pure culture on potato glycerine as described by Cayley (1921). The ascospores extruded were not suitable for measurement, since in most cases they had already germinated.

A number also were single-celled with few cell contents. Ascospores from perithecia developing on cankered bark under natural conditions have therefore been measured as described for macroconidia. The figures so obtained agree with those described for *Nectria galligena* Bres. The other characters of the fungus support this conclusion:

Perithecia: red, ovoid-pyriform, 300–450 × 250–350 μ, occurring singly or in groups. A stroma is not invariably apparent. Ostiole raised and consisting of radially arranged cells, of slightly darker colour. Asci: clavate; 8-spored, one spore occasionally abortive, 1- or 2-seriate, 85–120 × 10–16 μ. Ascospores: hyaline, 2-celled, often with constriction at the septum. Mean dimensions: 16·3 × 7·5 μ. Extremes: 11·5–22 × 5·5–9·2 μ.

Macroconidia: hyaline, 5–7-septate, allantoid. Abstricted from branched conidiophores aggregated into dirty-white sporodochia. Anastomoses frequent in old sporodochia. 3-septate, 39 × 5·1 μ; 4-septate, 47 × 5·3 μ; 5-septate, 60 × 5·6 μ; 6-septate, 69 × 5·9 μ; 7-septate, 71 × 6 μ.

Mycelium: hyaline with reddish stromata becoming dark brown. Inoculation of apple trees with pure cultures produced typical cankers.

(3) *Cultural characters.*

The fungus grows readily on the common media, forming much white aerial mycelium. After 15–20 days, cultures on malt-extract agar. potato-dextrose agar, and especially on oatmeal agar, developed plectenchymatous stromata which became dark reddish brown in colour. Wheat-meal agar produced very little mycelial growth. but conidial sporodochia appeared in a few days clustered around the inoculum. In appearance they closely resembled the sporodochia developing naturally on cankered bark. Similar sporodochia were formed on other media in the aerial mycelium, though their appearance was delayed in the cultures on potato glycerine (Cayley, 1921).

Measurement of the diameters of colonies of the fungus in pure culture on malt-extract agar at temperatures ranging from 2 to 37° C. showed that the optimum temperature for growth is about 20° C. Growth is slow and the hyphae stunted at 30° C. and the fungus is killed at 37° C. Growth is slow but appreciable at 2° C., and at this temperature the diameter of the colonies increased by about 0·8 mm. per day.

In pure cultures of the fungus on sterilized pieces of apple bark, conidial sporodochia developed and in course of time assumed the same colour as naturally occurring perithecia. No perithecia, however, were produced and the sporodochia thus modified were, at this stage, not able to form viable macroconidia. Structures resembling perithecia in size and colour were found in a Petri-dish culture of *N. galligena* on malt-extract

agar after about 3 weeks, but remained sterile. True perithecia with asci and ascospores developed in pure culture on the potato-glycerine medium (Cayley, 1921). The ascospores were found extruded at the ostioles of the perithecia, and many had germinated.

Anastomoses of conidia through short conjugation tubes as described by Cayley (1921) were of frequent occurrence in the sporodochia of older cultures. A similar though less frequent fusion was observed in the germ tubes of macroconidia germinating in hanging drops in distilled water.

(4) *Seasonal spore development.*

During late winter or early spring many small cankers may develop by infection at the leaf scars of the previous season's wood, as described by Wiltshire (1921). During a moderately warm spell in March or April, these small cankers may show the conidial sporodochia. These burst through the epidermis as dirty white circular cushions 1–2 mm. diameter, frequently arranged in roughly concentric arcs round the source of infection. Like the similar but looser sporodochia formed in culture, these consist of closely branching hyphae, from the tips of which the characteristic spores are abstricted. Sporodochia have been observed before the middle of March on young cankers resulting from leaf-scar infections. The conidia from these sporodochia were mostly two-celled however in place of the typical 5- or 6-septate spores.

Except in rather damp conditions, few sporodochia are produced during the summer months, but renewed activity is apparent in September and early October. The most favourable conditions are a warm humid spell following rain. True sporodochia are not formed after November, although macroconidia with 3–5 septa have been collected in small numbers throughout the year on vaselined glass slides.

Lower temperatures are followed by the appearance of the red perithecia, which at first develop at the edges of the sporodochia. The numbers of perithecia increase from October onwards, being at a maximum during December. Mature asci and ascospores are found on sectioning perithecia at the end of October or early November. Zeller (1926) for Oregon, U.S.A., conditions describes the time of maturing of the perithecia as the three months following the autumn rains. A continuous humid atmosphere from the beginning of October onwards resulted in spore discharge at the end of November or beginning of December. A dry autumn, however, caused perithecial development to be delayed, so that spore discharge was not observed until the first week in January.

Low temperature alone is not responsible for the formation of perithecia. Cultures of the fungus on sterilized pieces of apple bark were kept out of doors during the autumn and early winter of 1936. The conidial sporodochia became coloured in the same way as the perithecia of *N. galligena* but no trace of perithecial structures was observed on sectioning. Macroconidia were present but incapable of germination. Perithecia were found by Cayley (1921) to develop in culture on a medium rich in starch and in the presence of glycerine. Under natural conditions perithecia are much more numerous on old-established cankers than on small cankers on young wood.

In December, and especially later in the winter, white masses of ascospores may be observed at the ostioles of the perithecia. These masses, as they dry out, become yellowish in colour. They may appear on perithecia kept in moist conditions in the laboratory. Zeller (1926) has suggested that they arise from the simultaneous discharge of several asci blocking the ostiole of the perithecium. If the masses are removed, as by washing, normal ascospore discharge may be renewed for a time. Sections of perithecia bearing such spore masses frequently show more or less disorganization of the asci, which may be absent, ascospores with branching germ tubes lying free within the perithecium. It would appear, therefore, that the appearance of masses of ascospores at the ostiole may be an indication of the exhaustion of the perithecium.

SPORE DISSEMINATION

Ascospore discharge

(1) *Mode of discharge.*

As stated above, the present investigation has been directed towards determining the output of spores from the fructifications of the fungus throughout the year. Attention has been chiefly focused on the ascospores. Most accounts of the fungus in the past have described discharge of the ascospores from the perithecia by extrusion from the ostioles as small masses or tendrils. Wiltshire (1919), however, mentioned that it was convenient to establish pure cultures of the fungus directly from ascospores ejected on agar plates from perithecia placed below.

It has been observed that the ascospores of the fungus may be ejected from the perithecia with considerable force. The eight spores from each ascus are typically ejected simultaneously as a single mass which may be collected on a cover-glass or microscope slide (Pl. XXIII, fig. 1). Many separate groups, each containing eight ascospores, may thus be

deposited. Such groups have been collected in the laboratory on cover-glasses supported on glass rings at distances of up to 3 cm. vertically above perithecia of *N. galligena*. The perithecia employed were growing on detached portions of cankered apple bark placed on wet filter paper. Zeller (1926), in Oregon, also describes this forcible ejection of ascospores of *N. galligena* from the perithecia. Observations have demonstrated that under field conditions also, dispersal of the ascospores occurs predominantly by the forcible discharge of the eight spores of the ascus
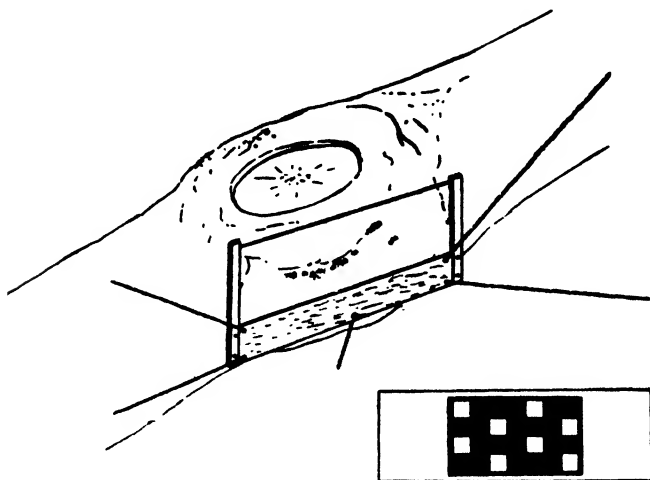
Text-fig. 1. Slide carrier and counting plate.

simultaneously. Figs. 1 and 2, Pl. XXIII, were photographed from vaselined slides used in the field work as described below, and fig. 2 shows clearly how the spores from two asci have crossed on the slide.

(2) *Field investigations.*

The perithecia of the fungus develop, frequently from the margin of the sporodochia, during the early autumn, becoming more numerous as winter approaches. Cayley (1921) follows previous workers in describing the perithecia as maturing during the winter and dehiscing in spring. Accordingly, preliminary spore-trapping experiments were started in the spring of 1936 in the plantations of Long Ashton Research Station.

(*a*) *Methods.* Vaselined 3 × 1 in. microscope slides were suspended on edge in suitable carriers (Text-fig. 1) close to cankers showing a number of perithecia. After suitable intervals—two or more days—the exposed

slides were brought into the laboratory, being replaced by fresh vaselined slides. The number of ascospores of *N. galligena* present in an area on each slide of $2 \times \frac{7}{8}$ in. was counted after adding sufficient aqueous solution of a proprietary wetting agent to allow clear visibility when a cover-glass was placed over the vaseline film. Note was also kept of the amount of daily rainfall. This procedure was maintained from March 1936 until the middle of May. The results showed that, in general, dispersal of ascospores from the perithecia was dependent on rainfall, but since the numbers fell away steadily from an initial maximum, it was decided to repeat the experiment the following year, commencing observations rather earlier.

In order to permit of more frequent counts of the ascospores, small squares distributed over the exposed vaselined slides were searched. This was achieved by superimposing the exposed slides on an engraved microscope slide placed on the microscope stage. This engraved slide was divided into 5 mm. squares, eight of which were searched. Text-fig. 1 explains their distribution. The total number of spores counted on each slide therefore represents those on an area of 2 sq. cm. If the number of ascospores exceeded about 800 per 5 mm. square, it was found advantageous to estimate the total from counts made on smaller squares delimited by means of a cross-hatched disk in the eyepiece of the microscope.

(*b*) *Records of seasonal and daily discharge.* Observations were commenced soon after the middle of October 1936 in the same plot of trees (var. Lane's Prince Albert) as had been used in the preliminary experiments. Four stations were selected for slides at distances of from 1·5 to 3 cm. from cankers showing numerous perithecia. After being exposed for one or more days, the four slides were replaced with fresh vaselined slides and taken to the laboratory for counting. Slides were changed in this way at 11 a.m., and the air temperature simultaneously recorded at the same height above ground as the cankers. Wet-bulb temperatures close to one of the cankers were also recorded, but the measure of humidity so obtained showed no relation to ascospore discharge. Daily rainfall was also recorded.

Recording was continued with four slides from October 1936 to June 1937, and thereafter with three slides until November 1937. The results are shown in Text-figs. 2 and 3. Text-fig. 2 represents the totals for 15-day periods from October 1936 to October 1937 of the ascospores of *N. galligena* collected on the slides from two of the slide stations recorded. The numbers of ascospores counted on the other slides show a similar relation to the time of year, but are not included since it was not found
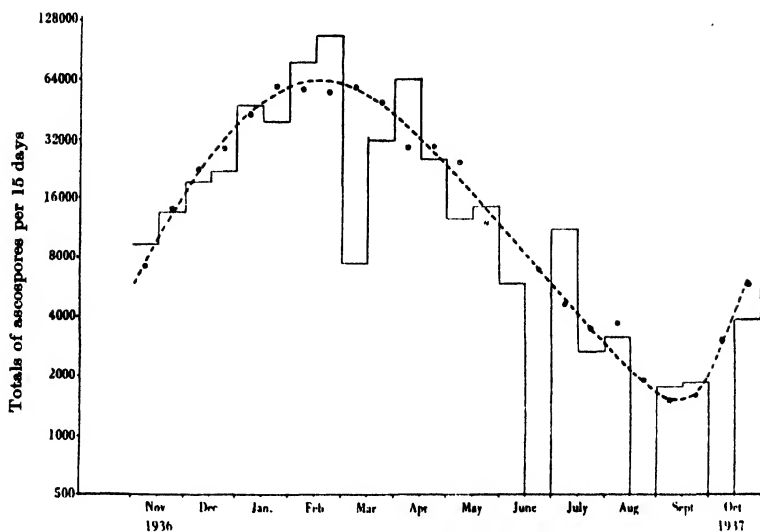
Text-fig. 2. Summarized records of ascospore discharge.
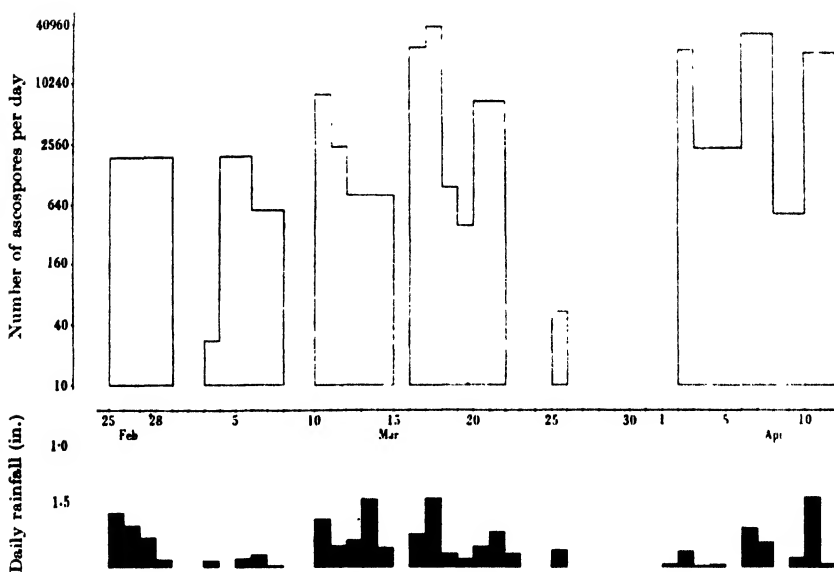
Text-fig. 3. Detailed records of ascospore discharge.

possible to maintain their records with sufficient consistency. The 15-day totals are shown as a stepped curve. In an effort to smooth out minor variations resulting from short wet periods or small temperature changes, means have been calculated for a series of 75-day periods, the middle point of each period being 15 days later than that of the preceding period. For each period the figure taken has thus been the arithmetic mean of the five 15-day totals included, and is plotted on the curve at the date representing the middle of the 75-day interval. Thus, the third point represents the arithmetic mean of the five 15-day totals extending from the beginning of November to the middle of January, the fourth, of the five totals from mid-November to the end of January, and so on. These means lie very near a smooth curve showing a distinct optimum period for ascospore discharge during the month of February, the numbers thereafter falling to a minimum during September, and then rising more steeply through October and November.

Over shorter intervals of time the numbers of ascospores collected show a close correlation with the presence or absence of rainfall. The detailed records for the period 25 February to 12 April 1937 are represented in Text-fig. 3, expressed as the totals for the four slides for each 24 hr. period. Where the slides were not changed daily the figures are given as the arithmetic mean per 24 hr. over the period of the exposure. There appears a very close correlation of presence of rainfall with ascospore discharge. In only one case was precipitation in excess of 0·02 in. not attended by discharge of ascospores, viz. from 22 to 23 March. It should be mentioned that, for this interval, the 0·11 in. recorded consisted almost entirely of snow accompanied by low temperatures, and thus the precipitation recorded on the graph for this date does not represent true rainfall. The actual numbers of ascospores discharged show no strict relation to the amount of rainfall for a particular interval of time, though the fact that such a relation exists is strongly suggested, bearing in mind the conditions of the experiment. A number of factors remain uncontrolled. These include, first, the actual time of wetting of the perithecia, which is influenced by wind direction, period of rainfall and relative humidity of the atmosphere, and, secondly, changes in the number of mature perithecia, and exhaustion resulting from prolonged discharge of ascospores.

(3) *Laboratory investigations.*

It was not possible to assess the effect of temperature on ascospore discharge under field conditions. At no time did rain fall when the

temperature was below 5° C. An attempt was therefore made to determine from laboratory experiments if ascospores were discharged at low temperatures. Groups of 30–40 perithecia of *N. galligena* (mature as far as could be judged) on small pieces of cankered bark were wetted and enclosed in Petri dishes at constant temperatures. The ejected ascospores were collected on the under side of cover-glasses supported a few millimetres above the perithecia by glass rings. At intervals the cover-glasses were removed in order to count the ascospores collected, fresh cover-glasses being substituted. The results indicated that, at 2° C., ascospore discharge is considerably reduced, by comparison with that at ordinary temperatures.

Groups of perithecia on cankered bark were wetted and left, as described, overnight at a constant temperature of 2–3° C. After 24 hr. seventy-five ascospores were counted. The same perithecia were then transferred to 18° C., when in 4 hr. over 1600 spores were collected. A similar experiment with perithecia at 2, 5 and 11° C. showed that discharge of ascospores was perceptibly slower at 2 and at 5 than at 11° C. Discharge may continue from a group of perithecia fairly uniformly over 48 hr.

Discharge of conidia

Naturally occurring sporodochia of *N. galligena* consist of closely interwoven branching hyphae, from the tips of which the conidia are produced. Under dry conditions they are compact structures of almost horny consistency, but in contact with water they swell rapidly, liberating many hundreds of conidia. No extensive observations of the dissemination of macroconidia of the fungus have been made, but parallel with the counts of ascospores collected on vaselined slides the numbers of macroconidia have also been counted. The numbers declined from an initial maximum in October 1936, and, as with the ascospores, no conidia were collected in absence of rainfall.

Macroconidia were rarely seen singly on slides placed below and beside cankers bearing sporodochia, but in groups whose disposition on the slide indicated that they had arisen by drops of water striking the slide. It is probable that, apart from being washed down the branch, such splashing represents the only method of dissemination of macroconidia. Wiltshire (1914) found that while woolly aphids carried conidia of the canker fungus, inoculation of the fungus through this agency was unsuccessful.

SPORE GERMINATION

Ascospores

The discharged ascospores are not enclosed in any kind of membrane. The contents of the ascus, with which they are ejected, are readily dispersed in water, but in the absence of a film of water, this material dries, fastening the spores to the substratum, from which they may then be washed only with difficulty. Ascospores collected on a clean glass surface and allowed to remain out of doors for 24 hr. are not removed completely by a vigorous washing under the tap.

The ascospores germinate rapidly in distilled water or nutrient media. Germ tubes are pushed out from the apex of one or both cells of the spore, or they may arise laterally. Germination has most conveniently been examined in the ascospores collected on cover-glasses, as described in connexion with the work on ascospore discharge. These cover-glasses were then transferred when desired, to be placed, spores downward, on glass rings in Petri dishes with damp filter paper. In the course of a few minutes water condensed on the lower side of the cover-glasses in sufficient amount to produce small hanging drops which grew round the spores.

In this way spores were obtained undamaged and, excluding the influence of the host plant, comparable with natural infective material. Germination will occur under such conditions through a temperature range of 2–30° C. Above 30° C. the spores are killed: at 25° C. spores germinate readily but the germ tubes are shorter than those appearing at 20° C. In many cases germination of the ascospores was about 95 % or more. The percentage was occasionally lower, however, owing to the presence of ascospores which were probably immature or abortive when discharged from the ascus. Not infrequently such spores were devoid of septa and had little or no protoplasm.

Counts were made, by the method indicated above, of the percentage germination of ascospores of the fungus at 25, 20, 15 and 2° C. The results are indicated in Text-fig. 4 as the means of a number of percentages, each of 100–300 ascospores. At 25° C. germination commenced in 2–3 hr. and was complete in 6–8 hr. At lower temperatures the rate of germination was reduced, though at 2° C. no actual inhibition of germination occurs. The final percentage of spores germinating at 2° C. is reached only after 4–5 days, but is as great as at higher temperatures. At 15° C. germ tubes up to 300μ in length were present after 24 hr.

The effect of desiccation on the germination of ascospores was tested by allowing the cover-glasses, with the spores adhering, to remain, protected from dust, in the atmosphere of the laboratory. At intervals these were set up for germination as described above. The few observations made indicated that the resulting percentage germination falls off steadily to zero after desiccation for 5–6 days under these conditions. Similarly, the percentage germination in the ascospores occurring in the spore masses at the ostioles of perithecia is usually less than the figure for freshly discharged ascospores.
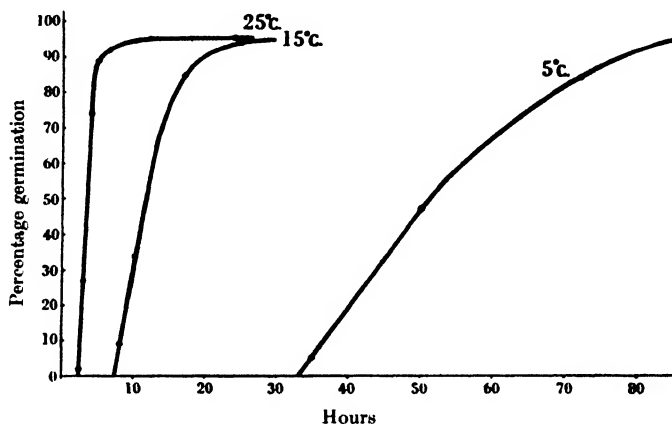
Text-fig. 4. Germination of ascospores of *Nectria galligena*.

Conidia

The macroconidia also germinate readily in distilled water and in nutrient media. Germ tubes appear at first usually from one or both terminal cells, though all the cells may ultimately show one or more germ tubes. With macroconidia from sporodochia on apple bark kept in moderately damp conditions germination may reach 100 %. Attempts were made, by adding small portions of the cortex of suitable apple varieties to the water in which macroconidia were germinating, to discover if resistant varieties exercised any inhibiting influence on spore germination. Cox's Orange Pippin, Lane's Prince Albert and Kingston Black (canker-susceptible varieties) were compared in this way with Bramley's Seedling and Sweet Alford (canker-resistant). Control conidia in distilled water gave a mean germination percentage of 88. Conidia in cortical extracts, on the other hand, germinated to not less than 98 % in every case.

DISCUSSION

The examination of the seasonal dissemination of ascospores and conidia of *Nectria galligena* reveals that discharge is not limited to a relatively short seasonal period, such as has been demonstrated for the ascospores of the scab fungus *Venturia inaequalis* (Keitt & Jones, 1926). The discharge of *V. inaequalis* ascospores, under American conditions, can be predicted with fair accuracy, so that protective measures can be concentrated within the limited period of maximum effectiveness.

With *Nectria galligena*, however, ascospore discharge remains at a high level in all rainfall periods from November to April or May. During the summer, relatively few spores are present but, in the autumn, conidia are produced in large numbers while ascospore output continues to increase. The ascospores are, for the most part, forcibly expelled from the perithecia to a distance sufficient to expose them to the dispersing influence of air currents. Even if the mechanism for forcible discharge breaks down and the ascospores accumulate at the ostiole, these masses may yet become dispersed, as shown by their occasional appearance on the slides of the spore trapping experiments in the field. Ascospore discharge ceases when the perithecia are no longer saturated, but is not retarded by cold until the temperature falls below 5° C.

Germination of the spores occurs under favourable conditions in a few hours and will take place over a temperature range of 2–30° C. Growth of the vegetative mycelium also takes place slowly at 2° C. in culture. A fungus able to maintain spore discharge and growth at relatively low temperatures, and to continue production of spores throughout the autumn, winter and spring has clearly unusual potentialities for infection of the dormant tree.

It is generally recognized that canker of apples is of much greater importance in the west than in the east of England. It is probable that this is due to climatic factors. The present investigation has emphasized that rainfall is necessary for the dissemination of the spores of *N. galligena*. It is also probable that the observation, due to Zeller (1926), that dry weather delays formation and maturing of the perithecia, will also be applicable in the east of England.

The very large numbers of ascospores collected on the vaselined slides make clear the potential infective power of cankered wood in relation to adjacent shoots and to neighbouring trees. The prompt removal of all such sources of infection is therefore of great importance in controlling the disease.

SUMMARY

1. The fungus causing apple canker is described and its identity with *Nectria galligena* Bres. is confirmed.

2. The methods of dispersal of the spores of *N. galligena* have been examined. The ascospores are discharged forcibly from the perithecium, the eight spores of each ascus typically being ejected simultaneously.

3. Field investigations have shown that spores are discharged during wet periods at all times of the year. Ascospore discharge reaches its maximum in January–February and falls to a minimum in the summer months.

4. The dispersal of conidia occurs during wet periods in the spring and autumn.

5. Germination both of ascospores and conidia occurs over a range of temperature from 2 to 30° C., the optimum being at 20° C.

REFERENCES

ALLESCHER, A. (1892). In *Ber. bot. Ver. Landshut,* 12, 130.
APPEL, O. & WOLLENWEBER, H. W. (1910). Grundlagen einer Monographie der Gattung *Fusarium. Arb. biol. Abt. (Anst.-Reichsanst.), Berl.,* 8.
BRESADOLA, J. (1901). In Strasser, *Pilzfl. Sonntagbl.* 4, 413.
CAYLEY, D. M. (1921). Some observations on the life history of *Nectria galligena* Bres. *Ann. Bot., Lond.,* 35, 79.
DICKSON, B. T. (1925). *Diseases of Fruit Crops in Quebec: Apple Diseases.* Macdonald College, Quebec.
DILLON-WESTON, W. A. R. (1927). Notes on the canker fungus. *Trans. Brit. mycol. Soc.* 12, 5.
GOETHE, R. (1904). *Über den Krebs der Obstbäume.* Berlin.
HARTIG, R. (1880). Der Krebs der Laubholzbäume. *Untersuch. forstbot. München,* 1, 209.
VON HÖHNEL, F. & WEESE, J. (1910). Zur Synonymie in der Gattung *Nectria. Ann. mycol., Berl.,* 8, 464.
KEITT, G. W. & JONES, L. K. (1926). Studies of the epidemiology and control of Apple Scab. *Res. Bull. Wis. agric. Exp. Sta.* no. 73.
MOORE, M. H. (1933). Some field observations on Apple Canker. *Ann. Rep. East Malling Res. Sta.* 1933, p. 166.
MORITZ, O. (1930). Studien über Nectriakrebs. *Z. PflKrankh.* 40, 251.
OGILVIE, L. (1935). The fungus flora of apple twigs and branches and its relation to apple fruit spots. *J. Pomol.* 13, 140.
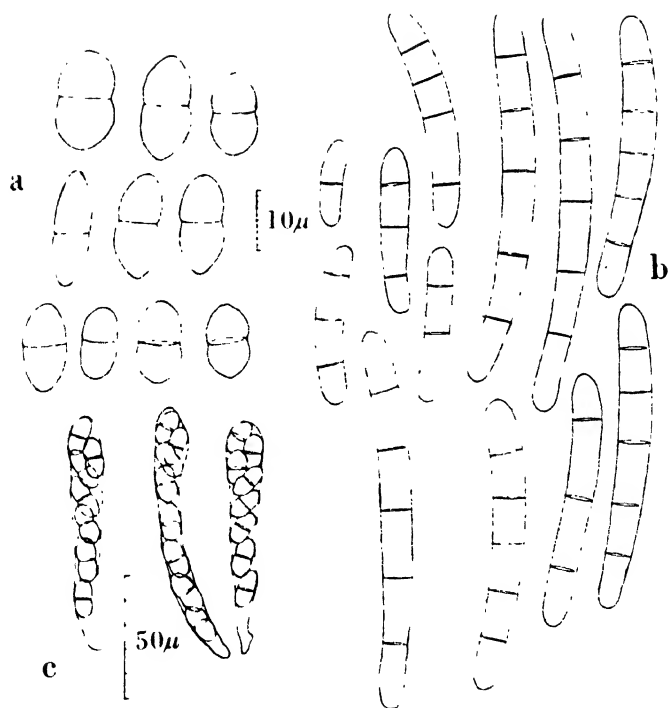
Fig. 3.

Osterwalder, A. (1931). Die *Nectria*-Kelchfäule an Äpfeln. *Gartenbauwiss.* **5**, 469.

Richter, H. (1929). Die wichtigsten holzbewohnenden Nectrien aus der Gruppe der Krebserreger. *Z. Parasitenk.* **1**, 24.

Tulasne (1865). *Selecta fungorum carpologia*, **3**, 73. Paris.

Weese, J. (1911). Zur Kenntniss des Erregers der Krebskrankheit. *Z. landw. Vchsw. Öst.* 1911, p. 872.

Westerdijk, J. & van Luijk, A. (1924). Untersuchungen über *Nectria coccinea* und *N. galligena*. *Meded. phytopath. Lab. Scholten*, **6**, 3.

Willkomm, M. (1866). *Die microscopischen Feinde des Waldes*, p. 103. Dresden.

Wiltshire, S. P. (1914). A note on the relation between Woolly Aphis and canker. *Ann. Rep. agric. hort. Res. Sta., Long Ashton*, 1914, p. 94.

—— (1919). The Apple Canker fungus. *Ann. Rep. agric. hort. Res. Sta., Long Ashton.* 1919, p. 23.

—— (1921). Studies on the Apple Canker fungus. I. Leaf-scar infection. *Ann. appl. Biol.* **8**, 182.

Wollenweber, H. W. (1913*a*). Studies on the *Fusarium* problem. *Phytopathology.* **3**, 24.

—— (1913*b*). Abgrenzung von Pilzgruppen. *Phytopathology*, **3**, 197.

—— (1929). Über Fruchtformen der krebserregenden Nectriaceen. *Z. Parasitenk.* **1**, 138.

Zeller, S. M. (1926). European canker of pomaceous fruit trees. *Bull. Oregon agric. Exp. Sta.* no. 222.

EXPLANATION OF PLATE XXIII

Figs. 1 and 2. Groups of *N. galligena* ascospores caught on vaselined slides in the field. Fig. 1 × 232. Fig. 2 × 182.

Fig. 3. Spore stages of *N. galligena*: *a* ascospores, *b* conidia, *c* asci.

OBSERVATIONS ON APPLE CANKER

II. EXPERIMENTS ON THE INCIDENCE AND CONTROL OF SHOOT INFECTIONS[1]

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(With 2 Text-figures)

CONTENTS

I. INTRODUCTORY

THE records given by Munson (1939) show that inoculum of *Nectria galligena* is present in the field throughout the year, being specially abundant during the winter and spring. Any spraying programme for protecting apple shoots from canker infection is therefore likely to be difficult and costly and, in dealing with mature trees, it is probable that the principal method of combating canker will continue to be by cultural treatments. The treatment generally recommended is grassing down, designed to check susceptibility to canker by reducing excessive vigour. Nursery material and other young trees, however, present a special problem in that strong growth is highly desirable in the early years of tree development. Further, young apple trees are readily accessible and valuable in relation to their size; consequently they may merit special

control treatments which would not be justifiable on older trees. For these reasons, this series of canker investigations has been started by a consideration of infections on trees not more than five years planted.

The researches of Wiltshire (1914, 1921, 1922) revealed the morphological details of canker infection of shoots through leaf scars. apple-scab lesions and woolly-aphis galls. Infections may also occur through major bark disruptions such as those due to mechanical injury or pruning cuts. Since in young trees the majority of cankers arise around leaf scars and pruning cuts, infections through these two channels were chosen for special study.

II. INOCULATION EXPERIMENTS

(1) *On leaf scars*

Wiltshire (1919) and Wiltshire & Spinks (1920) record that in a batch of trees obtained by crossing the two cider varieties Medaille d'Or and Kingston Black the leaf-scar infections were not seen before the spring. However, in the varieties King of the Pippins and Quarrenden. leaf-scar infections of the 1920 wood were found as early as 23 September 1920. In the present investigation the attempt was made to determine. for the variety Cox's Orange Pippin, the variation in susceptibility to leaf-scar infection throughout the dormant season.

The material utilized comprised forty 2-year-old trees of Cox's Orange Pippin potted in December 1936 and kept out of doors except during freezing weather when they were moved into a cool greenhouse. All the trees were transplanted into open ground on 18 March 1938. Six series of experiments were carried out on the following dates: 26 November 1936, 29 January 1937, 5 April 1937, 4 October 1937, 5 January 1938 and 14 March 1938. For each series of experiments forty 1-year shoots were selected of which half were sprayed as described in a later section.

The twenty sprayed shoots, together with the corresponding twenty unsprayed shoots, in each series, were inoculated, without wounding, by placing a fragment of agar bearing mycelium of *N. galligena* on one of the leaf scars, the inoculation being then covered with a crêpe rubber bandage. In the 1936–7 experiments all inoculations were made on the same day as the sprayings, but in the 1937–8 series. half of the inoculations were made on the day of spraying and half a week later. The crêpe rubber bandages were removed from the inoculations after a fortnight and the shoots were examined for canker infections at frequent intervals. The results of the inoculations are given in Table II.

It will be noted that infection in the winter months was practically nil under the conditions of this experiment. In October 1937, six trees out of the twenty unsprayed became infected and, in April 1937, sixteen out of the twenty. Trees inoculated in the spring of 1938, however, did

not become infected, but at that time the trees received a serious check through being transplanted on 18 March during a period of frost and drought which continued until the end of April. Previous observations have suggested that trees thus checked are highly resistant to canker infection.

(2) *On pruning cuts*

Cankers arising at pruning cuts are commonly noted in the field in the spring and early summer, but no records have been found of experiments made in this country to determine the time at which infections of these pruning wounds originate. Zeller (1926) in Oregon noted that infections following pruning took place most readily in the autumn. It has been
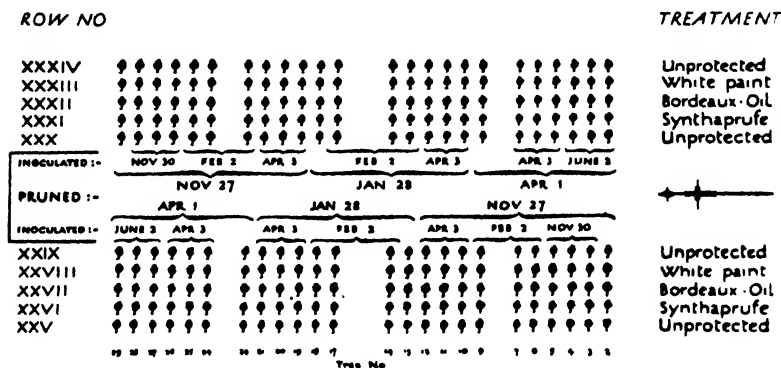
Fig. 1. Lay-out of wound protectant trial 1936-7.

shown by Munson (1939) that there is no periodic break in ascospore discharge from the autumn to the spring, so that the possibility of any variation in the susceptibility of the tree is of special interest. Further points chosen for investigation were the age of the wound in relation to resistance to infection and the merits of certain wound protectants.

The trees available for experiment comprised 240 Cox's Orange Pippins planted out in 1934-5 in ten rows of equal length (nos. XXV-XXXIV) running north and south (Fig. 1). To reduce the influence of tree position the treatments carried out on the western half of the block (rows XXV-XXIX) were repeated on the eastern half (rows XXX-XXXIV) in the reverse order. Experiments were carried out in each of the seasons 1935-6, 1936-7 and 1937-8. A typical lay-out is illustrated in Fig. 1, which gives a plan of the 1936-7 trial. It will be noted that 100 of the trees were pruned in November, seventy in January, and seventy in April, and that in each block ten trees remained uninoculated. The remainder of the November-pruned trees were inoculated, thirty within 1 week of pruning, thirty 2 months later and thirty 4 months

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later. Similarly, the January-pruned and April-pruned trees were inoculated in two batches; one shortly after pruning, the other 2 months later.

The pruning treatment applied prior to 1937–8 was a shortening of a single leader on each tree, the terminal wound being used for treatment. In 1937–8, one secondary shoot on each tree was cut flush at its point of intersection with a 2-year-old leader, thus leaving a lateral wound. If the wounds were to be protected, the protectant material was applied immediately after wounding. The inoculations, made after the

Fig. 2. (a) Results of wound protectant trial 1936–7. (b) Results of wound protectant trial 1937–8. Each square represents an inoculation: each black square represents a canker.

prescribed time, were carried out by placing fragments of an agar culture of *N. galligena* in contact with the protected or unprotected wound. The inoculation was then bound round with crêpe rubber which was removed a fortnight later. The 1935–6 season was used for a preliminary test of methods and materials: the results of the 1936–7 and 1937–8 trials are given in diagrammatic form in Fig. 2.

Considering first the unprotected pruning cuts, it is evident from these results that there was no period from October to March at which *N. galligena* mycelium failed to infect newly-made wounds. Infections initiated in December commonly required 6 months in which to become evident, but those starting in autumn or spring were plainly visible in 2 months. The age of the cut, i.e. the length of time elapsing between wounding and infection, was, however, of outstanding importance, and the great majority of infections arose from the inoculations made directly

after wounding. As the pruning cuts aged they acquired resistance to infection, and when the interval between wounding and inoculation was 2 months or longer, the proportion of successful infections was commonly low. An exception occurred in the pruning cuts of March 1938, which remained relatively susceptible in May of that year.

The effects of the wound protectant treatments will be considered in a later section, but it will be evident from Fig. 2 that, in general, the susceptibility or resistance of the cuts treated with these materials followed the same course as with the unprotected wounds.

III. CONTROL MEASURES

It has previously been noted that two important channels of canker infection are leaf scars and pruning cuts. As it is impracticable to treat leaf scars individually, they can be protected only by spraying the whole tree. The effectiveness of such protection will obviously be influenced by the wetting and spreading properties of the spray and by the tenacity of the fungicidal residue. Pruning cuts, however, may merit individual attention by the painting on of a protectant material, this application being restricted to the cut surfaces. The protectant paint may depend for its effectiveness either on toxicity to fungus spores, or on the presentation of a mechanical barrier. or on a combination of these factors.

The preparation of sprays and of paints which would be satisfactory in both fungicidal value and physical properties was made the subject of preliminary trials, followed by field experiments.

(1) *On leaf scars*

Laboratory tests. There have been numerous observations that spraying with lime sulphur does not directly control attacks by *N. galligena.*

In the present investigation, laboratory tests were carried out in which lime sulphur at a number of concentrations was atomized on to slides under standardized conditions to give a deposit of 0·008 ml. of spray/sq. cm. After being allowed to dry, the slides were sown with drops of a water suspension of ascospores of *N. galligena.* Comparable experiments were made with Bordeaux mixtures of different copper concentrations. The results are given in Table I, each figure being the mean percentage germination obtained by counting a total of 600 spores in six replicated drops.

Table I shows that even under conditions more favourable to the fungicide than those found in the field, lime sulphur was non-toxic to *N. galligena* spores at 1/11 strength and only partially toxic at 1/3·7. A mixture of 1 % lime sulphur with 3 % grade G petroleum oil was also found to be non-toxic. Sprays containing lime sulphur were therefore

Table I. *Toxicity of lime sulphur and of Bordeaux mixture to ascospores of* Nectria galligena

| | % germination of *N. galligena* ascospores on slides sprayed with | |
G. fungicide/l. of spray	Lime sulphur	Bordeaux mixture
0·11	—	56
0·33	—	43
1·0	—	35
1·1	97	—
3·0	—	17
3·3	97	—
9·0	—	5
10·0	93	—
30·0	96	—
90·0	98	—·
270·0	42	—
810·0	0	-

omitted from further consideration, while Bordeaux mixture was chosen to form the fungicidal basis of a spray application.

Field trials. Wiltshire & Spinks (1920) record that spraying with copper stearate in December 1919 gave favourable results in controlling leaf-scar infections, but that a spray with 4–4–50 Bordeaux in November 1921 was unsuccessful. Moore (1933) states that Bordeaux spraying over a period of 5 years tended to reduce canker infection on Cox's Orange Pippin.

The prime requirements of a protectant spray for use on apple shoots are a high level of retention and of tenacity. It has been shown by Fajans & Martin (1938) that these requirements are best met by an oil-emulsifier-solid system with a minimum quantity of emulsifier. To provide a complete cover of the leaf scars on an apple shoot it is, however, necessary that the spray used should have adequate wetting and spreading power, which calls for an increase of the proportion of emulsifier in the oil-emulsifier-solid spray. After a number of preliminary tests on apple shoots the compromise reached in this investigation was a spray made up as follows:

Copper sulphate	8 lb.
Hydrated lime	16 lb.
Casein	4 lb.
Petroleum oil	1 gal.
Water	100 gal.

In this mixture the lime and the oil both provided tenacity, the casein conferred wetting power and the oil spreading power. Because of its wetting ability, the initial retention of the spray was relatively low.

In the present investigation spraying trials were carried out for 2 years in autumn, winter and spring on the trees of Cox's Orange Pippin previously described in the section on leaf-scar inoculation experiments. No infections, either on sprayed or unsprayed trees, resulted from the inoculations made in November 1936, January 1937 and January 1938. Of the twenty unsprayed trees inoculated in April 1937, sixteen trees became cankered, while in the corresponding sprayed series six showed

Table II. *Results of leaf-scar inoculation experiments*

No. of shoots	Date of spraying	Date of noculation	No. of infections
	1936	1936	
20	26 Nov.	26 Nov.	0
20	—	26 Nov.	0
	1937	1937	
20	29 Jan	29 Jan.	0
20	—	29 Jan.	0
20	5 Apr.	5 Apr.	6
20	—	5 Apr.	16
10	4 Oct.	4 Oct.	1
10	4 Oct.	11 Oct.	0
10	—	4 Oct.	1
10	—	11 Oct.	5
	1938	1938	
10	5 Jan.	5 Jan.	0
10	5 Jan.	12 Jan.	0
10	—	5 Jan.	0
10		12 Jan.	0
10	14 Mar.	14 Mar.	0
10	14 Mar.	21 Mar.	1
10	—	14 Mar.	0
10	.	21 Mar.	1

In addition, two uninoculated shoots developed cankers in spring 1937.

infection. In October of the same year, twenty unsprayed trees showed six infections as compared with one in the sprayed series. The full results are given in Table II.

(2) *On pruning cuts*

Materials. The practice of protecting pruning cuts has been much debated, and a summary of work on wound protectants is given by Howe (1915) who concludes that there is nothing to be gained by their use on apple wood. A recent paper by Moore (1933) records that uninoculated wounds on Cox's Orange Pippin wood remained free from canker whether treated with neat tar distillate, bituminous emulsion, colloidal sulphur, white lead paint or no protectant. Zeller (1926) recommends the treat-

ment of wounds by a paint made by mixing a proprietary copper-lime dust with linseed oil.

The list of materials tested in the present investigation is as follows:

A. Oil-Bordeaux made up of

copper sulphate	...	...	...	8 g.
hydrated lime ...	...	...	...	16 g.
casein ...	...	...	...	4 g.
petroleum oil (grade G)		...	...	10 ml.
water ...	...	...	...	1 l.

B. Cuprous oxide powder as specified by Horsfall (1938) in one-third its weight of edible cotton-seed oil.

C. Copper-lime-linseed oil consisting of

monohydrated copper sulphate		...	5 g.
hydrated lime ...	...	...	10 g.
boiled linseed oil	...	...	9 ml.

D. Commercial white lead paint[1] (white lead, conforming to B.S.S. no. 239, comprising 60 % of total solids).

E. "Synthaprufe" proprietary bituminous emulsion in liquid and paste forms.

F. Soft grafting wax (proprietary product).

G. Mercury-glycerine wound dressing (see Day, 1924) consisting of

mercuric cyanide	...	...	...	6·25 g.
mercuric chloride	...	...	...	6·25 g.
glycerine	...	...	...	3 l.
water ...	...	...	...	1 l.

In addition, tests were made of a number of Bordeaux pastes (copper sulphate 65 g., lime 130 g., water 1 l.) with and without cotton-seed oil and linseed oil.

Laboratory tests. Ascospores of *N. galligena* sown on slides painted with either Synthaprufe or white lead paint showed vigorous germination, the germ tubes growing freely alongside and through both paints. The oil-Bordeaux-casein mixture, in a comparable test, completely inhibited spore germination, but this material leaves a deposit which is too tenuous to be suitable for wound protection. Similarly, the mixtures of copper sulphate, lime, water and oils proved too free-flowing for field application. It appeared to be essential to add a dry material to the oil to obtain a paint of the required consistency, and a number of mixtures were made up similar to the paint recommended by Zeller (1926). The formula finally adopted is given above (C).

Further laboratory trials of the fungicidal value of certain of these materials were carried out by painting them on to the upper cut ends of

[1] Not the specially thickened paint recommended by Brooks & Moore (1925).

detached shoot portions, then placing *N. galligena* spores in a drop of water on the painted surface, the whole being enclosed in a moist chamber. After 24 hr. the spores were examined for germination. Of the materials mentioned above, only the mercury-glycerine dressing and the copper-lime linseed oil were entirely effective in preventing spore germination in these circumstances. The mercury dressing was found to be injurious to young shoots of apple and was therefore not employed in field trials.

Field trials. Field trials were carried out each winter from 1935 to 1938 on the 240 trees of Cox's Orange Pippin described in an earlier section. Certain materials which had not proved satisfactory in laboratory tests, but which were often applied in practice, were included for purposes of comparison. The complete list of materials tested on a field scale is as follows:

White lead paint; soft grafting wax; "Synthaprufe" bituminous emulsion; Bordeaux oil; cuprous oxide in cotton-seed oil; copper-lime-linseed oil. Full descriptions of these materials are given in a preceding section.

The technique of wounding has already been described, and a typical lay-out for the wound protectant trials is shown in Fig. 1. On all cuts to be protected, the protectant material was applied immediate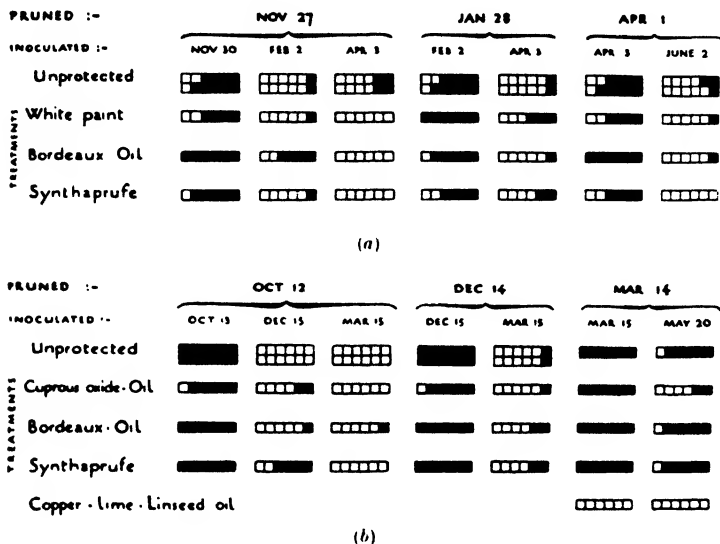ly after wounding. The inoculations were then made after the prescribed times by placing fragments of an agar culture of *N. galligena* on the wound and binding round with crêpe rubber which was removed a fortnight later.

It has previously been mentioned that the 1935–6 season was used for a preliminary test of materials and methods and, as one result of this trial, soft grafting wax was discarded as being too difficult to manipulate on small wounds. The results of the 1936–7 and 1937–8 experiments are presented in diagrammatic form in Fig. 2.

It must be emphasized that the wound protectants used were subjected to a very stringent test, a relatively large mass of fungus mycelium supplied with nutrient being held closely applied to the treated wound for a fortnight. In these circumstances, wounds treated with all but one of the materials tested became cankered as readily as the wounds left unprotected. The exception was the copper-lime-linseed oil mixture which, in a limited number of field tests, gave complete control in spite of the onerous conditions of the experiment.

Observations concerning the direct effect of the various wound protectants on the tissues of the apple shoots, confined to the variety Cox, showed that the cuprous oxide oil mixture caused local necrosis of the

bark and a marked browning of the wood. This discoloration, which was associated with the presence of gum in the xylem vessels, extended up to 4 in. from the painted surface. In the wounds treated with white paint, with Synthaprufe and with Bordeaux-oil there was delay in callusing and a shrinkage and cracking of the bark tissues surrounding the treat cut. The copper-lime-linseed oil appeared to have no adverse effect on callusing, and the cuts treated healed over as quickly as comparable non-infected and unpainted wounds.

IV. DISCUSSION

It has been shown that lime-sulphur and tar-oil spraying have no effect on the production of *N. galligena* spores, while neither petroleum oil nor lime sulphur, at strengths used in the field, has any effect on their germination. The use of Bordeaux mixture on apples in this country has now been largely discontinued because of its tendency to cause fruit russeting when applied at the "pink" and "petal fall" stages. Unfortunately, this tendency is shown most seriously on the canker-susceptible dessert apples, Cox's Orange Pippin, James Grieve and Worcester Pearmain. The present-day routine spraying programme for apples—tar oil in December or January, petroleum oil in March, and lime sulphur in spring and summer—thus makes no direct contribution to the control of canker.

A consideration of the spore discharge records shows that the likelihood that shoot infections will be initiated during the summer months is low, so that spraying during this period will have only a minor effect on the incidence of canker. Again, the inoculation experiments on leaf scars indicate that unwounded shoots do not readily become infected during December or January. For this reason the addition of fungicides to the tar-oil wash with the object of controlling canker does not seem likely to achieve success. The critical periods for leaf-scar infection are in the spring and autumn. In the present paper an instance is given of the beneficial effect of a Bordeaux-casein-oil spray, applied in April, in reducing canker infection. Such a spray could be introduced into the routine programme by adding Bordeaux mixture to the petroleum oil emulsion now applied in March. Alternatively, Bordeaux mixture could be substituted for lime sulphur in the first pre-blossom or "green flower" scab spray. However, the combined Bordeaux-petroleum spray would be preferable, since the presence of the oil would improve wetting and sticking properties.

In the autumn, the protection of trees against canker infections of

leaf scars could not be achieved without including a spray application outside the present routine programme. A spray with Bordeaux mixture, preferably mixed with an oil emulsion, merits consideration for nursery stock and young trees of canker-susceptible varieties. It should be applied as soon as possible after leaf fall.

Turning to the problem of pruning-cut infections, it is noteworthy that dry and cold conditions are unfavourable to the discharge and germination of the spores of *N. galligena*, thus suggesting an advantage in choosing spells of dry frosty weather for pruning operations. Another factor concerning time of pruning is the prolonged susceptibility of wounds made in March. This phenomenon, taken together with the rising spring temperatures favouring spore germination, indicates that late pruning is prejudicial to canker control.

The behaviour of the pruning cuts made in March forms an exception to the general rule that wounds 2 months old or more show a natural resistance to infection. This feature of the results dealing with the inoculation and protection of pruning cuts necessitated a new approach to the problem of wound treatment. Much previous work on this subject has been directed to the provision of a weatherproof seal for large wounds which would give mechanical protection for a long period—possibly several years. Sealing materials of low fungicidal value, however, proved ineffective against inoculations with *N. galligena*, and numerous observations have shown that they are not highly effective against canker in general orchard practice. For a pruning cut of relatively limited size, made during the winter months, the requirement for canker control is a covering which will prevent disruption of the surface and maintain an effective concentration of fungicide on the wound for the period before natural resistance is established. The mixture of monohydrated copper sulphate with hydrated lime, compounded to a thick paint with linseed oil, shows promise of fulfilling this requirement without causing injury to the shoots or interfering with callusing, but large-scale trials with the material are necessary before its value can be established.

V. Summary

1. Inoculations with *N. galligena* on unwounded shoots of apple show that canker infections through leaf scars may be initiated in October and April but not in November and January. Shoots sprayed before inoculation with a Bordeaux-casein-oil mixture gave a much lower proportion of infection than those unsprayed.

2. Inoculations with *N. galligena* on pruning cuts showed that freshly made pruning cuts on apple shoots are susceptible to canker infection throughout the dormant season. Pruning cuts made during autumn and winter acquire a natural immunity to infection within 2 months.

3. Several wound sealing materials applied to pruning cuts failed to give protection from canker when the treated surfaces were inoculated with *N. galligena*. The most promising wound protectant tested was a mixture of monohydrated copper sulphate, hydrated lime and linseed oil.

REFERENCES

BROOKS, F. T. & MOORE, W. C. (1925). Silver-leaf disease. V. *J. Pomol.* **5**, 61–97.

DAY, L. H. (1924). Experiments in control of cankers of fire blight. *Phytopathology*, **14**, 478–80.

FAJANS, E. & MARTIN, H. (1938). The incorporation of direct with protective insecticides and fungicides. III. Factors affecting the retention and spray residue of emulsions and combined emulsion-suspensions. *J. Pomol.* **16**, 14–38.

HORSFALL, J. G. (1938). Combating damping-off. *Bull. N.Y. (Geneva) agric. Exp. Sta.* no. 683.

HOWE, G. H. (1915). The effect of various dressings on pruning wounds of fruit trees. *Bull. N.Y. (Geneva) agric. Exp. Sta.* no. 396.

MOORE, M. H. (1933). Some field observations on apple canker. *Ann. Rep. East Malling Res. Sta.* 1933, pp. 166–75.

MUNSON, R. G. (1939). Observations on apple canker. I. The discharge and germination of ascospores of *Nectria galligena*. *Ann. appl. Biol.* **26**, 440–457.

WILTSHIRE, S. P. (1914). A note on the relation between woolly aphis and canker. *Ann. Rep. agric. hort. Res. Sta., Long Ashton*, 1914, p. 94.

—— (1919). The apple canker fungus. *Ann. Rep. agric. hort. Res. Sta., Long Ashton*, 1919, pp. 23–9.

—— (1921). Studies on the apple canker fungus. I. Leaf-scar infection. *Ann. appl. Biol.* **8**, 182–92.

—— (1922). Studies on the apple canker fungus. II. Canker infection of apple trees through scab wounds. *Ann. appl. Biol.* **9**, 275–81.

WILTSHIRE, S. P. & SPINKS, G. T. (1920). Apple tree canker. *Ann. Rep. agric. hort. Res. Sta., Long Ashton*, 1920, pp. 82–3.

ZELLER, S. M. (1926). European canker of pomaceous fruit trees. *Bull. Oregon agric. Exp. Sta.* no. 222.

PHYTOPHTHORA ERYTHROSEPTICA PETHYBR. IN RELATION TO ITS ENVIRONMENT

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(With 2 Text-figures)

CONTENTS

INTRODUCTION

IN 1929 the writers commenced a study of pink rot of the potato and, later, published their observations on the disease (Cairns & Muskett, 1933 a). The present account describes an investigation made with the object of obtaining a clearer conception of the role played by the more salient environmental factors in the physiology of the normal pathogene, *Phytophthora erythroseptica* Pethybr., and in the incidence of the disease caused by this organism.

ENVIRONMENTAL FACTORS

Staling

It has been shown by Brown (1925) and others that, if a fungus is grown in a medium where its staling products tend to accumulate, the resultant staling effect on the growth of the organism may be pronounced. In view of this, observations were made on the staling effect by (a) plotting a periodic growth rate, and (b) comparing the rates of growth in different depths of the same medium.

The daily growth rate of the fungus in plates of potato-mush and malt-extract agars at 22° C. was recorded by measuring the diameters of the colonies, the measurements being continued until the plates were fully covered (see Fig. 1). Comparison of the rates of growth in different depths of the same medium at 22° C. was made on oat and malt-extract agars, the measurements being taken when the fungus had grown almost to the edges of the plates (see Table I).
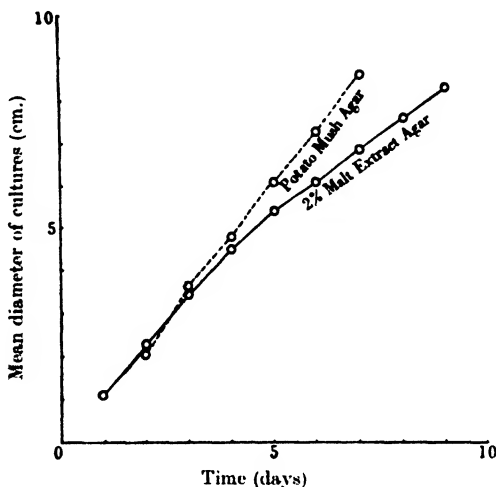
Fig. 1. Growth rates of *P. erythroseptica* at 22° C. on 2 % malt-extract agar and on potato-mush agar.

Table I

		Mean diam. (5 plates) cm.
Oat: Plates containing 15 c.c. medium		8·2
„ „ 45 c.c. „		7·8
Malt: „ „ 10 c.c. „		7·8
„ „ 40 c.c. „		7·6

In all the *natural* media used in the present work the fungus grows, apparently at a constant rate, up to the edge of the plate, and it is evident that the staling effect is negligible on these media. No allowance was therefore made for staling in the subsequent work where these media were employed.

Nevertheless, a constant growth rate does not occur in all media; it has been noted that the rate of growth decreases with age and ceases about 1 cm. from the edge of the plate in cultures on certain synthetic media and notably where potassium nitrate is used as a source of nitrogen.

Light

The following are details of an experiment on the effect of light on the growth and reproduction of *P. erythroseptica*.

Two groups, each of seven plates of malt-extract agar, were inoculated and incubated at laboratory temperature. The plates of one group (*a*) were placed on a white surface near a large window, so that they might be exposed to daylight, while at the same time they were further exposed to the continuous light of a 100 W. lamp placed at a distance of 2 ft. above them. The other group (*b*) was enclosed in a well-aerated light-proof chamber, which consisted of a double box arrangement with two opposite air vents in each box, the vents of one box alternating with those of the other. Layers of black paper were loosely placed round the plates for further protection. The mean daily temperature was approximately 17° C. in group *a*, and 16° C. in group *b*, while the average growth measurements after 10 days were

Group *a*. Exposed to continuous light (17° C.)   ...   8·4 cm.

Group *b*. Continuous darkness (16° C.)   ...   ...   7·7 cm.

These results were confirmed by a parallel series grown in potato-mush agar.

It thus appears that light has no appreciable effect on the growth rate of the fungus. The small difference in growth rate obtained may be explained by the temperature difference of 1° C. which occurred under the varying conditions.

Numerous oospores were produced under both conditions, and to apparently the same extent. They were particularly profuse in the potato-mush agar. No sporangia were found.

The effect of light on the production of sporangia in liquid media was not studied in detail, but on different occasions these were observed in cultures exposed to the light of the laboratory and also in those which had been kept in dark incubators. Waterhouse (1931) did not find light to be concerned in the production of sporangia in the several species of *Phytophthora* which she studied.

Acidity

In a provisional investigation of the relationship of *P. erythroseptica* to this factor, two media—2 % malt-extract agar and 15 % potato-mush agar (raw tuber 150 g./l.)—were prepared. Each medium was then divided into parts and sterilized, after which the reaction was adjusted in each part to give series ranging by steps of 0·2–1·0 from *p*H 2·5 to 11·0. Each medium was poured immediately without further sterilization. No supplement of a buffer substance was given. On cooling, the plates were inoculated from cultures grown on media adjusted to a corresponding reaction. They were then incubated at 18° C.

The results showed that the acid limit for growth was *p*H 3·2, and that the optimum covered a wide range in the region *p*H 6·0–7·0, but because

of the acid drift the alkaline limit could not be ascertained. For example, a malt medium of pH 10·0, in which the fungus had become established and grown well, had dropped to pH 8·4 during the experimental period.

Oospores were produced freely over the greater part of the pH growth range but not towards the limits of acidity or alkalinity. Sporangia were not observed on any of these gel media.

Like staling and light it was apparent that this factor need not be taken into account in the experiments dealing with moisture and temperature.

Moisture

Reproduction. Pethybridge (1913) found that sexual reproduction in *P. erythroseptica* occurs freely in gel- and liquid-media, but asexual reproduction is limited to liquid. This has been confirmed in the present work. Both Pethybridge (1913) and Murphy (1918) observed sporangia developed occasionally in drops of condensed moisture on aerial hyphae, but this has never been the experience of the writers. More recently Leonian (1934) has shown how asexual reproduction may be readily induced by aquatic conditions following culture of the organism in a highly nutrient pea medium; and McKay (1937) finds that older cultures (6–12 months) produce sporangia more readily than younger cultures (1 month).

Infection. Although typical pink rot of the potato is normally caused by *P. erythroseptica*, or more rarely by *P. megasperma* (Cairns & Muskett, 1933 *b*). a somewhat similar condition of the tuber may result from simple asphyxiation such as would be caused by the flooding of land carrying a potato crop. This is assumed by many farmers who commonly refer to the disease as "Waterslain" or "Drowning". Work carried out under aseptic conditions in this laboratory has shown that the death of the tuber, or the lowering of its resistance to such an extent that it becomes readily invaded by putrefactive organisms, occurs after submergence in water for a period of at least 24 hr. (temp. 16–20° C.). The decline of the tuber by such asphyxiation is accompanied by symptoms, which although somewhat atypical, bear a close resemblance to those of pink rot resulting from parasitic causes. In so far as *P. erythroseptica* is concerned in the causation of pink rot, excessive soil moisture is also conducive to the incidence of the disease, in which case the infection of the tuber is frequently *not* via the mother stolon.

The writers have frequently observed in cases where tubers in storage have contracted the disease and where infection has occurred after their detachment from the mother plant, that the infection often occurs at the

"rose" end of the tuber. They have further shown by experiment that tubers may become directly affected with the disease when placed in moist soil artificially contaminated with the parasite. The general results of an experiment of this nature which has already been described (Cairns & Muskett, 1933 *a*) are as follows:

(1) Of 241 tubers stored in moist soil contaminated with *P. erythroseptica*, eighty-eight became infected with pink rot.

(2) Fifty-six of these eighty-eight tubers, where they were only partly affected, showed initial attack at the "rose" end, the diseased area usually radiating from an "eye". The soil in this experiment was of approximately the maximum moistness provided by capillarity from basal pans, and the temperature was that of January–February out-of-doors.

In order to obtain more information regarding the conditions governing direct infection of the tuber, the following experiment was undertaken.

A number of healthy potato tubers were washed and kept in a moist atmosphere for about 4 days in order to allow the healing of any wounds caused by washing. A glass ring, approximately 2·0 cm. in diameter and 1·0 cm. deep, was then fitted to each of the tubers by means of sealing cement and paraffin wax. The tubers were divided into two lots which were ringed as follows: (*a*) with the ring enclosing an "eye", (*b*) with the ring fitted on a portion of the tuber not involving an "eye". After ringing, the tubers were left for a further 2 days when the rings were filled with a water extract from the soil used in the previous experiment, and in each was placed a small inoculum (5–6 sq. mm.) of a 5–6 days old culture of *P. erythroseptica*. The tubers were then put in glass potato dishes and placed in an incubator at 22° C. The results obtained from this experiment were:

Variety	Locus of ring	No. of tubers	No. of tubers infected
Arran Victory	Internode	10	0
	"Eye"	7	6
Kerr's Pink	Internode	8	0
	"Eye"	11	7

With each of the above four lots a further two tubers were similarly treated but no *P. erythroseptica* was added. All of these control tubers remained healthy throughout the experiment.

The above results confirm those obtained by the preliminary trials and show that under conditions providing ample moisture, the healthy tuber readily contracts infection through the "eye". That such conditions are necessary is confirmed by the fact that in two further experiments where the inoculum was placed directly on the "eye" or the internodal region of the tuber without the use of a ring containing free water, no infection was obtained. Although the actual mode of infection was not observed, it was seen that sporangia were produced by the inoculum after its inclusion in the fluid within the ring.

Temperature

The effect of temperature has been investigated in relation to the growth and reproduction of the fungus and its infection of the potato tuber.

Growth. The relation of temperature to growth rate has been studied on oat-, potato- and malt-extract agars. The results obtained are presented in Fig. 2, where it will be seen that the temperatures for the
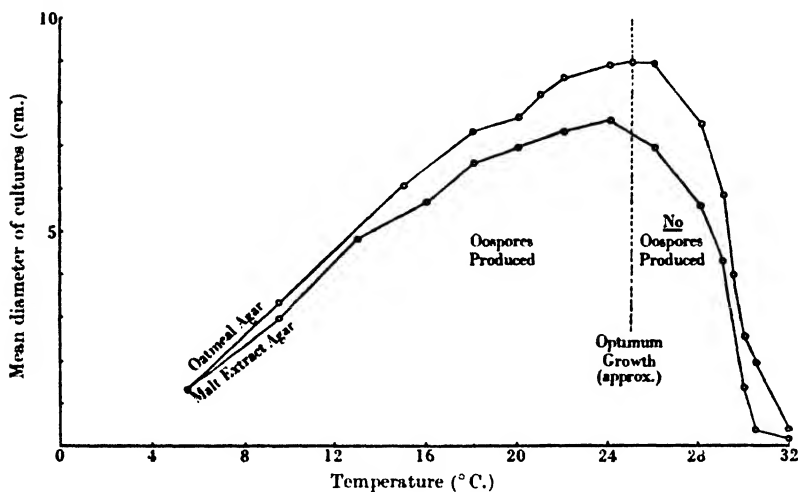
Fig. 2. Temperature-growth relationship for *P. erythroseptica* when growing on oatmeal agar and malt-extract agar.

minimum, optimum and maximum growth rates approximate 5, 25 and 31° C. respectively.

Infection. It has been observed by farmers that the incidence of pink rot in stored potatoes increases as spring succeeds winter, i.e. with a rise in the mean daily temperature. The following experiments were made to determine the effect of temperature upon direct infection of the potato tuber.

Five groups, each of twelve tubers (Arran Victory), were fitted with glass rings as previously described. In ten tubers of each group the ring was fitted so as to include an "eye", while in the remaining two it was fitted to the internodal region. The rings were filled with sterilized bog soil (peat) extract, and inoculations were made from a young culture of *P. erythroseptica* on oat agar. The tubers were then placed in glass potato dishes lined with moist absorbent paper, and each of the five groups incubated under different conditions of temperature. When infection had taken place and the

disease had progressed so far in a tuber that it could be identified by external symptoms, the tuber was removed and cut in half through the point of inoculation in order to obtain the confirmatory colour symptoms. The experiment (*a*) was allowed a duration of 21 days and was repeated (*b*) with a duration of 28 days. Details and results are given in Table II.

At the end of the experimental period the tubers of group A were transferred to a temperature of 20° C. for 14 days, when three tubers in A (*a*) and four tubers in A (*b*) had become affected with the disease through "eye" inoculation. Group E (*a*) was similarly treated, with the result that nine of the tubers inoculated at an "eye" contracted the disease.

Table II

Group	Storage conditions and temp. (' C.)		"Eye" inoculation	Internode inoculation	Time taken for disease to become obvious days
A	Refrigerator	(*a*) 5–7	0	0	—
		(*b*) 7–9	0	0	—
B	Laboratory	(*a*) 12–15	8	0	20
		(*b*) 17–20	9	0	16
C	Incubator	(*a*) 19–20	7	0	13
		(*b*) 20	6	0	7
D	Incubator	(*a*) 23–25	8	0	9
		(*b*) 24–25	9	0	10
E	Incubator	(*a*) 27–29	1	0	–
		(*b*) 30	0	0	· –

No. of infected tubers

The results of these experiments provide ample evidence of the relation between temperature and direct infection of the tuber. It would appear that below 10° C. and above 30° C. infection rarely takes place, and that in the intervening temperature range optimum conditions for infection and development of the disease occur in the neighbourhood of 20–25° C.

DISCUSSION

In any discussion of pink rot of the potato it must be borne in mind that the disease may be caused by organisms other than *P. erythroseptica*, and that rather similar symptoms may be produced in tubers the tissues of which have been killed through exposure to lethal substances or through prolonged submergence in water or waterlogged soil. In many cases, however, apart from the characteristic coloration, the symptoms are somewhat atypical where *P. erythroseptica* is not the pathogene. The present investigation, which is concerned solely with the normal pink rot pathogene, *P. erythroseptica*, has elucidated considerably the relationship of this organism to its environment, and affords a fuller conception of the mode of tuber infection in the course of farm practice.

Although in artificial media in Petri dishes there was no appreciable

decrease in the rate of growth of the fungus through accumulation of its *own* staling products, this may not be true in the soil where the antagonistic effect of other organisms may be very pronounced. It was found that, when growing in artificial media, the fungus was intensely intolerant towards contaminants in general.

Under soil conditions light would not be involved except in the surface layers and, when investigated experimentally, this factor produced no significant effect.

Modification of the relative acidity of artificial media showed that the limits for growth were pH 3·2 and *circa* pH 9·0–10·0, with an optimum in the region of pH 6·0–7·0. Sexual reproduction was confined to within the limits pH 4·0–8·0, with an optimum at pH 6·0–7·0; outside this range oospores were not found, even in 6-week-old cultures. Asexual reproduction was not induced by any pH in a gel medium, but this effect was not studied in liquid media in detail. In view of this tolerance of *P. erythroseptica* to a wide range of hydrogen-ion concentration, and if it be considered that the soil in the potato-growing lands of Northern Ireland has a general reaction of pH 5·0–6·5. and that the most favourable soil reaction for growth of the potato is within the limits pH 4·8–5·7 (Comber, 1927), then it is obvious that any modification of soil acidity to inhibit development of *P. erythroseptica* is impracticable.

In this connexion the addition of lime and acid to contaminated land on two occasions did not offer any conclusive evidence as to decrease or increase in disease intensity. Nevertheless, lime as a soil flocculant should assist in improving drainage and thus tend to limit the incidence of the disease.

Sporangia were only produced under aquatic conditions, an observation which may be correlated with the fact that moisture is of primary importance in cases of *direct* infection of the potato tuber such as occur in the pit or clamp. The experiments dealing with moisture and infection generally indicated this. particularly where rings containing soil solution were fitted on different *loci* of the tuber. These experiments also demonstrated that *direct* infection is normally via the "eye" of the tuber. Before digging, infection is normally via the stolon and is therefore *indirect*. In laboratory experiments tubers in contaminated soil remained free from infection when its moisture content was not higher than about 19 %, but where the moisture content was raised to 24 %, infection readily occurred. With soil at its maximum moisture content all tubers became readily affected with pink rot, but attack by *P. erythroseptica* under these conditions tended to become obscured by the

possibility of asphyxiation of the tubers and attack by putrefactive organisms. Where severe outbreaks of pink rot have been reported from farms it has been observed that the soil had been abnormally wet for a period of several days previous to the outbreak. On the other hand, it has been observed that, in land known to be contaminated with *P. erythroseptica*, the incidence of pink rot was negligible in a dry season. In a field test, in light, well-drained soil, fifty tubers were planted in ridges with a plate culture of *P. erythroseptica* and portions of pink rot tubers placed in close proximity to each; yet all the tubers gave rise to healthy plants and there was no pink rot in the crop, which was similar in quantity to that of the uncontaminated (control) plot.

The results of an investigation into the temperature relationships of *P. erythroseptica* demonstrated one point of particular interest, i.e. that sexual reproduction only occurs between the minimum and optimum temperatures for growth (5–25° C.). The effect of temperature on the production of sporangia was not examined critically; but infection of tubers was inhibited above 25° C.—an effect which seems to be related to the production of sporangia, assuming that zoospores are the agents of infection under such conditions. Normal summer soil temperatures of about 20° C. would be very favourable for the growth of, and infection by, the parasite.

Any correlation of soil temperatures with the temperature relationships of *P. erythroseptica* must be very general, yet there is obviously a definite relation between them. Taking as typical the monthly *average* temperatures for a soil depth of 1 ft. at Hillsborough, Northern Ireland, it is seen that over a period of 5 years (1930–5) these averaged during the potato-growing season: April 7·3° C., May 10·4° C., June 13·7° C., July 15·5° C., Aug. 15·1° C., Sept. 13·4° C. and Oct. 10·6° C. Potatoes are grown at a depth of much less than 1 ft. and are therefore subject in summer to an average temperature slightly higher than that recorded, coupled with a correspondingly greater diurnal variation. The curves of the Oxford values given by Keen & Russell (1921) for depths of 6 and 12 in. are similar, the temperature at the shallower depth being less than 1° C. higher in summer and 1° C. lower in winter. At the temperatures recorded at Hillsborough for the dead season—Nov. 7·6° C., Dec. 6·0° C., Jan. 5·1° C., Feb. 4·8° C. and March 5·1° C.—the growth of *P. erythroseptica* must be practically inhibited, the more so since the figures for the cultivated layer would be less than those given, which are for 12 in.

In general, therefore, with *P. erythroseptica* present in soil otherwise favourable to the fungus, it would appear that warm, moist weather

persisting for a period of several days is most likely to encourage the onset of pink rot in the potato crop. That this is so has been observed by the writers on several occasions, and it was particularly noticeable during 1936 when, during an abnormally wet July and August, vigorous crops became affected with pink rot within a short time. These observations on temperature and moisture are significant in relation to the statement made by Pethybridge (1913) to the effect that the disease is of much more importance in the west of Ireland.

Summary

1. The present contribution, as part of a general study of pink rot of the potato, deals with *Phytophthora erythroseptica* Pethybr. in its relation to staling, light, acidity, moisture and temperature.

2. In natural media the fungus is not, or but very slightly, affected by its own staling products, but, in general, is intensely intolerant to the presence of other fungi and bacteria.

3. Ordinary daylight is not concerned in the growth or reproduction of the fungus.

4. Growth occurs over a wide pH range (3·2 to *circa* 9·0–10·0); sexual reproduction occurs over this range except towards the limits of acidity and alkalinity; asexual reproduction was not investigated experimentally. It is considered that modification of the soil reaction, within practical limits, does not offer a means for control of the disease.

5. The moisture factor controls directly the incidence of the disease in contaminated soil—with *low* moisture infection is inhibited; with *normal* moisture infection occurs via the dead or moribund stolon through the "heel" of the tuber; with high moisture the tuber is infected directly in the region of an "eye". Sporangia are only formed under aquatic conditions and it is considered probable that "eye" infection is brought about through the agency of zoospores.

6. The temperature limits of growth are 0–32° C. with an optimum at 25° C. Oospore production occurs only between the optimum and minimum growth temperatures (0–25° C.), being inhibited above the growth optimum. Asexual reproduction seems to be similarly related to temperature.

REFERENCES

BROWN, W. (1925). Studies in the genus *Fusarium*. II. *Ann. Bot., Lond.*, **39**, 373.

CAIRNS, H. & MUSKETT, A. E. (1933a). Pink rot of the potato. *Ann. appl. Biol.* **20**, 381.

—— —— (1933b). *Phytophthora megasperma* causing pink rot of the potato. *Nature, Lond.*, **131**, 277.

COMBER, N. M. (1927). *An Introduction to the Scientific Study of the Soil*, p. 105. Edward Arnold and Co.

KEEN, B. A. & RUSSELL, E. J. (1921). The factors determining soil temperature. *J. agric. Sci.* **11**, 211.

LEONIAN, L. H. (1934). Identification of *Phytophthora* species. *Bull. W. Va. agric. Exp. Sta.* no. 262.

MCKAY, R. (1937). Potassium permanganate as an aid to the production of asexual fructification by *P. erythroseptica* Pethybr. *Nature, Lond.*, **139**, 802.

MURPHY, P. A. (1918). The morphology and cytology of the sexual organs of *Phytophthora erythroseptica*. *Ann. Bot., Lond.*, **32**, 115.

PETHYBRIDGE, G. H. (1913). On the rotting of potato tubers by a new species of *Phytophthora* having a method of sexual reproduction hitherto undescribed. *Sci. Proc. R. Dublin Soc.* **13** (N.S.), 529.

WATERHOUSE, G. M. (1931). The production of conidia in the genus *Phytophthora*. *Trans. Brit. mycol. Soc.* **15**, 311.

SKIN SPOT (*OOSPORA PUSTULANS* OWEN & WAKEF.) OF THE POTATO, AND ITS CONTROL BY TUBER DISINFECTION

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(With Plate XXIV and 1 Text-figure)

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I. INTRODUCTION

SKIN SPOT (*Oospora pustulans* Owen & Wakef.) is a disease which is not generally recognized by the farmer as causing damage to the potato tuber. As the lesions are superficial and do not extend to any great depth in the flesh, the loss in ware tubers with few exceptions is practically negligible. With seed potatoes the disease is of some consequence. as severe attacks, especially in the region of the eyes, may prevent sprouting and thus cause "misses" in the crops. Recent records in Great Britain (Ministry of Agriculture, etc. 1937), where losses of from 20 to 30 $^{0}/_{0}$ in stocks of seed have been noted, would suggest that the disease is of some importance. The nature of the occurrence of skin spot is also deceptive. A supply of seed purchased or selected in the autumn or early winter may confidently be stored as perfectly sound and apparently free from the disease. When examined in the spring, however, it may be discovered that the disease has developed during storage and that many of the tubers have failed to sprout. Such an occurrence may result in the planting of a smaller acreage than originally intended since, at that time of the year, it is often impossible to obtain a further supply of suitable seed. The planting of diseased seed may also result in "misses" in the crop.

The methods generally recommended for the prevention of losses due to skin spot are usually of a precautionary nature. The selection and

boxing of seed immediately after lifting, in conjunction with efficient and hygienic storage, will do much to reduce the incidence of the disease, while the rejection of non-sprouted or poorly sprouted tubers at planting time assists in preventing "misses" in the field. It was thought that a more direct method of control which would prevent the development of the disease should prove to be of considerable value, and it was with this aim that experimental work was commenced in 1933 to investigate the effect of tuber disinfection.

II. PRELIMINARY EXPERIMENTS

Experiments were made during 1933–5 to test the value of the disinfection of seed potatoes before planting for preventing the development of skin spot in the resultant crop during storage. The seed planted in all these experiments was selected as severely affected with skin spot, and was disinfected a few weeks before planting. Disinfection was carried out by immersing the tubers in solutions of various materials, the immersion period varying from short or "instantaneous" dips (0·5–1 min.) to steeps of 90 min. duration. In addition to mercuric chloride and formalin, two proprietary organo-mercury preparations A and B were employed. The potatoes were planted in land which had not previously borne a potato crop and the plots were randomized, each plot consisting of one drill 20 yd. in length. In one experiment (1933–4) the plots were duplicated. The number of sets planted per plot varied from thirty-five to forty. At the time of planting all the tubers had sprouted, and although a few "misses" did subsequently occur in most of the plots there was no evidence that these were due to skin spot or that disinfection had reduced their number.

The crops were lifted at the end of September or the beginning of October a few weeks after the haulms had completely died down. The small-sized chats, blighted and unsound tubers were discarded. The remainder of the crop from each plot was clamped immediately where it was thought that conditions would be most suitable for the development of skin spot, the tubers being covered with a layer of straw before "earthing up". The clamps were not opened until the following spring when all the tubers were examined for skin spot. They were divided into two classes, (1) affected with skin spot, and (2) not affected, any tubers showing one or more skin-spot lesions being classified as affected. The results of these experiments (Table I) show that all the disinfectants employed, with the exception of formalin, produced a definite reduction

Table I. *Control of skin spot by tuber disinfection, 1933–5 experiments*

Treatment		1933–4 Sharpe's Express		1933–4 Kerr's Pink		1934–5 Sharpe's Express	
		Total crop lb.	% affected with skin spot	Total crop lb.	% affected with skin spot	Total crop lb.	% affected with skin spot
Nil	a	54	81·5	—	—	—	—
	b	48	79·2	87	77·0	43·5	79·3
	Mean	51	80·4	—	—	—	—
0·1 % mercuric	a	58	56·9	110	22·7	42	50·0
chloride, 90 min.	b	51	33·3	—	—	—	—
steep	Mean	54·5	45·1	—	—	—	—
0·25 % organo-	a	54·5	53·2	—	—	—	—
mercurial A,	b	47·5	50·5	114	14·9	—	—
15 min. steep	Mean	51	51·9	—	—	—	—
0·75 % organo-	a	63·5	52·8	—	—	—	—
mercurial A, in-	b	48	20·8	—	—	—	—
stantaneous dip	Mean	55·8	36·8	—	—	—	—
1·0 % ,,		—	—	—	—	44	54·5
1·5 % ,,	a	58	43·1	—	—	—	—
	b	62	43·5	—	—	—	—
	Mean	60	43·3	—	—	—	—
1·5 % organo-mercurial B, instantaneous dip		—	—	—	—	51·5	56·3
Formalin (1 : 240), 90 min. steep		—	—	109	83·9	—	—

in the amount of skin spot which developed. It was also noted that the incidence or severity of the disease was generally much less on the affected tubers grown from disinfected seed than on those grown from non-disinfected seed. Consequently, the reduction in the amount of skin spot due to disinfection was greater than that indicated by the tabulated results.

III. The time of disinfection

Although the results from the preliminary experiments were promising, nevertheless a larger reduction in the incidence of the disease was desirable, and in order to study the effect of varying the time of treatment a fuller investigation was commenced in 1935. Here it was decided to compare the efficiency of disinfection at two distinct times, viz.:

(*a*) Immediately before planting, i.e. disinfection of the seed tubers in the spring, the resultant crop *not* being disinfected before storage.

(*b*) Immediately after digging, i.e. the planting of *non-disinfected* seed tubers and the disinfection of the resultant crop before storage.

Two varieties of potato, Kerr's Pink and Sharpe's Express, were used, all the seed being selected as severely affected with skin spot. For (*a*) the seed tubers were disinfected about 1 week prior to planting, while for

(*b*) the resultant crop was disinfected immediately after lifting. Three methods of disinfection were employed:

(1) A 90 min. steep in a 0·1 % mercuric chloride solution.

(2) An "instantaneous" dip in a 1·5 % solution of organo-mercurial B.

(3) An "instantaneous" dip in a 1·0 % solution of organo-mercurial C, a modified form of organo-mercurial A used in the preliminary experiments.

The planting, lifting and storage of the crop were similar to the technique adopted for the preliminary experiments with the exception that each plot. consisted of one drill 10 yd. in length, and that the same number of sets was planted in each plot. After storage in clamps during the winter the tubers were washed and examined for skin spot in the following spring (1936). They were then divided into three classes:

(*a*) Clean—those showing complete freedom from skin-spot lesions.

(*b*) Slightly diseased—those showing one to five skin-spot lesions per tuber.

(*c*) Severely diseased—those showing six or more skin-spot lesions per tuber.

The number and weight of tubers in each class were recorded, but as the data obtained in each case showed a close degree of correlation, tuber numbers only are recorded in Table II. Here the total number of tubers falling into classes (*a*), clean, and (*b*), slight skin spot, are also grouped together in a category designated as "commercially clean". It is quite possible that a few or even one lesion per tuber may in some cases cause severe injury to the young sprouts, and this may be particularly true when the infection is in the region of the eyes. In most of the tubers examined which belonged to the slight skin-spot class, however, the infection was observed at the "heel" end. Thus, for commercial purposes it would appear to be quite legitimate to regard such slightly affected tubers as "clean".

From these results it will be seen that disinfection of seed tubers a few weeks before planting again gave a definite reduction in the incidence of the skin spot which developed in the tubers of the resultant crop during storage. A much more efficient control of the disease, however, was obtained where the seed tubers were *not* disinfected but the resultant crop disinfected immediately after digging.

The efficacy of disinfection immediately after digging in preventing the development of skin spot during storage was confirmed during 1936–7. Potatoes of the varieties Arran Banner and Kerr's Pink grown on a farm in Co. Down from seed affected with skin spot and not disin-

Table II. Control of skin spot by tuber disinfection, 1935–6 experiment

Variety	Time of disinfection	Treatment		Total no. of tubers	Severe skin spot %	Slight skin spot %	Clean tubers %	Commercially clean %	Total skin spot %
Sharpe's Express	Seed tubers *not* disinfected, resultant crop disinfected immediately after digging	Water, instantaneous dip		113	65·5	30·1	4·4	34·5	95·6
		0·1 % HgCl₂, 90 min. steep		137	1·5	21·9	76·6	98·5	23·4
		1·5 % organo-mercurial B, instantaneous dip		120	0·7	26·7	66·6	93·3	33·4
		1·0 % organo-mercurial C, instantaneous dip		96	1·1	3·1	95·8	98·9	4·2
,,	Seed tubers disinfected before planting, resultant crop *not* disinfected after digging	Nil		154	41·6	45·5	12·9	58·4	87·1
		0·1 % HgCl₂, 90 min. steep		134	4·5	32·8	62·7	95·5	37·3
		1·5 % organo-mercurial B, instantaneous dip		151	22·5	31·8	45·7	87·5	54·3
		1·0 % organo-mercurial C instantaneous dip		165	4·9	30·3	64·8	95·1	35·2
Kerr's Pink	Seed tubers *not* disinfected, resultant crop disinfected immediately after digging	Water, instantaneous dip		169	90·5	7·7	1·8	9·5	98·2
		0·1 % HgCl₂, 90 min. steep		108	28·5	30·5	41·0	71·5	59·0
		1·5 % organo-mercurial B, instantaneous dip		150	36·0	31·3	32·7	64·0	67·3
		1·0 % organo-mercurial C, instantaneous dip		168	0·6	4·8	94·6	99·4	5·4
	Seed tubers disinfected before planting, resultant crop *not* disinfected after digging	Nil	a	161	78·9	14·9	6·2	21·1	93·8
			b	161	78·2	14·3	7·5	21·8	92·5
			Mean	161	78·6	14·6	6·8	21·4	93·2
		0·1 % HgCl₂, 90 min. steep	a	176	35·8	38·1	26·1	64·2	73·9
			b	128	38·3	48·4	13·3	61·7	86·7
			Mean	152	37·0	43·3	19·7	63·0	80·3
		1·5 % organo-mercurial B	a	165	38·1	36·4	25·5	61·9	74·5
			b	102	29·0	30·9	40·1	71·0	59·9
			Mean	103·5	33·6	33·6	32·8	60·4	67·2
		1·0 % organo-mercurial C	a	203	29·5	35·5	35·0	70·5	65·0
			b	250	26·8	42·4	30·8	73·2	69·2
			Mean	226·5	28·2	38·9	32·9	71·8	67·1

fected before planting were harvested during October 1936. Quantities of ware and seed size of each variety were forwarded immediately to the Agricultural Research Institute, Hillsborough, where they were disinfected on the day after digging. After disinfection the tubers were thoroughly dried and stored. Two methods of storage were employed: (1) in a clamp, (2) in sprouting boxes in a potato house. In the clamp the experiment was arranged in duplicate, while in the sprouting boxes it was carried out in triplicate. The quantities of tubers used and the treatments given were as follows:

I. Clamp storage
2 lots each 0·5 cwt.—no treatment—Control.
2   „   „   „   "Instantaneous" dip in 1·5 % organo-mercurial B.
2   „   „   „   "Instantaneous" dip in 0·5 % organo-mercurial C.

II. Stored in sprouting boxes
3 lots each 21 lb. (i.e. 3 boxes)—no treatment—Control.
3   „   „   „   „   „   "Instantaneous" dip in 1·5 % organo-mercurial B.
3   „   „   „   „   „   "Instantaneous" dip in 0·5 % organo-mercurial C.

The examination for skin spot was made during the following May and June, the tubers being divided into three classes as in 1936. The results recorded in Tables III and IV show that tuber disinfection immediately after digging gave excellent control of skin spot. They also show that in the case of the non-disinfected tubers the incidence of severe skin-spot attack was much higher under the conditions of clamp storage than in the sprouting boxes. Disinfection also appears to be somewhat less efficient for tubers stored in sprouting boxes, this being especially marked in the slight skin-spot class.

Many of the tubers stored in the clamp were affected with a bacterial soft rot, probably due to attack by *Bacillus carotovorus* either directly or indirectly, with *Fusarium* rot, pit rot or blight (*Phytophthora infestans*) as forerunners. A count of the number of rotten tubers in the various lots indicated that disinfection had given a promising control. All the rotten tubers were discarded and were not included in the skin-spot results. Very few rotten tubers were present in the sprouting boxes and all the tubers were well sprouted.

That immediately after digging is the most satisfactory time for

Table III. *Control of skin spot by tuber disinfection,*
Kerr's Pink, 1936–7 experiment

Method of storage	Treatment		Total no. of tubers	Severe skin spot %	Slight skin spot %	Clean tubers %	Commercially clean %	Total skin spot %
Clamp	Nil	a	195	52·3	17·9	29·8	47·7	70·2
		b	86	72·1	14·0	13·9	27·9	86·1
		Mean	140	62·2	16·0	21·8	37·8	78·2
	1·5 % organo-mercurial B, instantaneous dip	a	232	9·9	14·2	75·9	90·1	24·1
		b	226	12·4	19·5	68·1	87·6	31·9
		Mean	229	11·2	16·8	72·0	88·8	28·0
	0·5 % organo-mercurial C, instantaneous dip	a	183	2·2	7·1	90·7	97·8	9·3
		b	203	1·0	5·9	93·1	99·0	6·9
		Mean	193	1·6	6·5	91·9	98·4	8·1
Sprouting boxes	Nil	a	92	47·8	28·3	23·9	52·2	76·1
		b	95	45·3	33·7	21·0	54·7	79·0
		c	101	35·6	46·5	17·9	64·4	82·1
		Mean	96	42·9	36·2	20·9	57·1	79·1
	1·5 % organo-mercurial B, instantaneous dip	a	90	25·6	35·6	38·8	74·4	61·2
		b	91	12·1	31·9	56·0	87·9	44·0
		c	92	27·2	30·4	42·4	72·8	57·6
		Mean	91	21·6	32·6	45·8	78·4	54·2
	0·5 % organo-mercurial C, instantaneous dip	a	89	3·4	18·0	78·6	96·6	21·4
		b	94	6·4	26·6	67·0	93·6	33·0
		c	78	1·3	17·9	80·8	98·7	19·2
		Mean	87	3·7	20·8	75·5	96·3	24·5

disinfection was again demonstrated in an experiment carried out during 1937–8, the results of which are shown in Table V. In addition to comparing the efficacy of various fungicides and confirming results previously obtained, one of the objects of this experiment was to determine the efficacy of tuber disinfection at various intervals after digging for the control of skin spot during storage. Tubers of the variety Kerr's Pink severely affected with skin spot were planted in land which had grown potatoes during 1935 and 1936. The 1935 crop was severely affected with the disease. Unfortunately, the 1936 crop was not retained in storage for skin-spot examination and, consequently, there is no record of the degree of attack on the tubers for this year.

In 1937 randomized plots each of one drill 9 yd. in length were used for the work and the various treatments, details of which are given in Table V, were carried out in duplicate. All the plots were harvested on 1 November, the produce of those requiring immediate treatment being disinfected on the same day. The tubers were stored in sprouting boxes for 3 days after treatment in order to ensure thorough drying, after which they were removed to a clamp. In the clamp the produce of the individual plots was separated by a thick layer of straw. The lots which

Table IV. *Control of skin spot by tuber disinfection,*
Arran Banner, 1936–7 experiment

Method of storage	Treatment		Total no. of tubers	Severe skin spot %	Slight skin spot %	Clean tubers %	Commercially clean %	Total skin spot %
Clamp	Nil	a	120	79·2	15·0	5·8	20·8	94·2
		b	134	74·6	14·9	10·5	25·4	89·5
		Mean	127	76·9	15·0	8·1	23·1	91·9
	1·5 % organo- mercurial B, instantaneous dip	a	180	6·1	16·7	77·2	93·9	22·8
		b	152	7·2	11·2	81·6	92·8	18·4
		Mean	166	6·7	13·9	79·4	93·3	20·6
	0·5 % organo- mercurial C, instantaneous dip	a	167	0·0	9·0	91·0	100·0	9·0
		b	175	1·1	4·6	94·3	98·9	5·7
		Mean	171	0·6	6·8	92·6	99·4	7·4
Sprouting boxes	Nil	a	85	30·6	35·3	34·1	69·4	65·9
		b	72	26·4	40·3	33·3	73·6	66·7
		c	87	20·7	32·2	47·1	79·3	52·9
		Mean	81	25·9	35·9	38·2	74·1	61·8
	1·5 % organo- mercurial B, instantaneous dip	a	71	9·9	26·8	63·3	90·1	36·7
		b	76	7·9	22·4	69·7	92·1	30·3
		c	76	17·1	21·1	61·8	82·9	38·2
		Mean	74	11·6	23·4	65·0	88·4	35·0
	0·5 % organo- mercurial C, instantaneous dip	a	70	0·0	12·9	87·1	100·0	12·9
		b	70	1·4	18·6	80·0	98·6	20·0
		c	71	1·4	16·9	81·7	98·6	18·3
		Mean	70	0·9	16·1	83·0	99·1	17·0

required disinfection at various intervals after digging were stored in a separate clamp from which they were removed as required, disinfected, and placed in the other clamp.

Examination for skin spot was made at the time of disinfection, but the disease was not observed until 8 weeks after digging when a few tubers were seen to be affected. Sixteen weeks after digging many tubers had become severely affected with the disease.

The clamp was opened in May 1938, and all the tubers washed and examined. The results, which are given in Table V, show that under the conditions of this experiment, the disinfection of the tubers 2 or 4 weeks after digging was as satisfactory in securing the control of skin spot during storage as when it was carried out on the day of digging. When a longer interval was allowed to elapse the results were not so satisfactory, and this is particularly marked where 16 weeks intervened between digging and treatment. It will also be seen that there were distinct differences in the efficiency of the various fungicides employed and, in this respect, the results largely confirm those obtained in previous experiments.

Table V. *Control of skin spot by tuber disinfection, 1937–8 experiment*

Time of disinfection	Treatment		Total no. of tubers	Severe skin spot %	Slight skin spot %	Clean tubers %	Commercially clean %	Total skin spot %
Seed tubers *disinfected* before planting. Resultant crop *not* disinfected after digging	Nil	a	171	44.4	31.5	24.1	55.6	75.9
		b	181	61.9	25.4	12.7	38.1	87.3
		Mean	176	53.1	28.5	18.4	46.9	81.6
	0.5 % organo-mercurial C, instantaneous dip	a	203	34.5	33.0	32.5	65.5	67.5
		b	179	17.3	26.8	55.9	82.7	44.1
		Mean	191	25.9	29.9	44.2	74.1	55.8
	1.5 % organo-mercurial B, instantaneous dip	a	181	29.3	26.5	44.2	70.7	55.8
		b	218	42.2	34.4	23.4	57.8	76.6
		Mean	199	35.8	30.4	33.8	64.2	66.2
	0.1 % mercuro-chloride, 90 min. steep	a	180	19.4	27.2	53.4	80.6	46.6
		b	205	19.0	31.2	49.8	81.0	50.2
		Mean	192	19.2	29.2	51.6	80.8	48.4
Seed tubers *not* disinfected. Resultant crop *disinfected* immediately after digging (i.e. on the same day)	0.5 % organo-mercurial C, instantaneous dip	a	176	0.0	2.8	97.2	100.0	2.8
		b	184	0.5	9.8	89.7	99.5	10.3
		Mean	180	0.3	6.3	93.4	99.7	6.6
	1.5 % organo-mercurial B, instantaneous dip	a	166	5.4	16.0	78.6	94.6	21.4
		b	181	7.2	19.3	73.5	92.8	26.5
		Mean	173	6.3	17.7	76.0	93.7	24.0
	0.1 % mercuro-chloride, 90 min. steep	a	183	3.3	14.2	82.5	96.7	17.5
		b	207	0.5	17.9	81.6	99.5	18.4
		Mean	195	1.9	16.1	82.0	98.1	18.0
	1.5 % formalin, instantaneous dip	a	153	27.5	36.6	35.9	72.5	64.1
		b	165	18.8	55.2	26.0	81.2	74.0
		Mean	159	23.2	45.9	30.9	76.8	69.1
	Proprietary dust applied at rate of 4 lb. per ton potatoes	a	170	45.3	26.5	28.2	54.7	71.8
		b	175	54.9	19.4	25.7	45.1	74.3
		Mean	172	50.1	23.0	26.9	49.9	73.1
Seed tubers *not* disinfected. Resultant crop disinfected at varying intervals after digging	2 weeks after digging, 0.5 % organo-mercurial C, instantaneous dip	a	170	1.2	7.6	91.2	98.8	8.8
		b	193	0.5	4.7	94.8	99.5	5.2
		Mean	181	0.8	6.2	93.0	99.2	7.0
	4 weeks after digging, 0.5 % organo-mercurial C, instantaneous dip	a	201	2.5	5.0	92.5	97.5	7.5
		b	171	1.2	7.0	91.8	98.8	8.2
		Mean	186	1.9	6.0	92.1	98.1	7.9
	8 weeks after digging, 0.5 % organo-mercurial C b	a	194	10.3	16.5	73.2	89.7	26.8
		b	211	4.7	11.8	83.5	95.3	16.5
		Mean	202	7.5	14.2	78.3	92.5	21.7
	16 weeks after digging, 0.5 % organo-mercurial C b	a	186	41.9	28.5	29.6	58.1	70.4
		b	187	20.3	28.9	50.8	79.7	49.2
		Mean	180	31.1	28.7	40.2	68.9	59.8

IV. THE TIME OF DIGGING AND INCIDENCE OF SKIN SPOT

During 1937–8 an experiment was carried out to determine whether the time of digging the crop would have any effect on the amount of skin spot which subsequently developed in storage. Seed tubers of the variety Kerr's Pink were selected as severely affected with skin spot, and on 17 May 1937 were used for the planting of twenty plots, each plot consisting of one drill of 9 yd. Twenty sets were planted in each plot. Five diggings were made at different dates during the season, the first

Block C	1st digging	5 August	3rd digging	1 October	Block A
	3rd ,,	1 October	5th ,,	29 November	
	4th ,,	1 November	2nd ,,	26 August	
	2nd ,,	26 August	4th ,,	1 November	
	5th ,,	29 November	1st ,,	5 August	
Block D	3rd ,,	1 October	5th ,,	29 November	Block B
	5th ,,	29 November	2nd ,,	26 August	
	1st ,,	5 August	4th ,,	1 November	
	4th ,,	1 November	3rd ,,	1 October	
	2nd ,,	26 August	1st ,,	5 August	

Text-fig. 1.

on 5 August when the tubers were small and immature, and while the foliage was still growing vigorously. The fifth and last digging was made on 29 November, the haulms having completely died down about 7 weeks previously. Skin spot was not observed on the tubers during any of the diggings. In October 1936 the disease was, however, noted by the writers at the time of lifting tubers of the same variety.

The lay-out of the plots and the dates of digging are given in Text-fig. 1. For each digging four of the plots were lifted, one plot being selected at random from each of the four blocks A, B, C and D. The small-sized chats, blighted and unsound tubers were discarded. The produce of the two plots from blocks A and C were thoroughly mixed and then divided into two lots of equal weight. One lot was disinfected immediately, an "instantaneous" dip in 0·5 % organo-mercurial C being employed, while the second lot was not disinfected. The produce of the two plots from blocks B and D received similar treatment. After disin-

fection all the tubers, disinfected and non-disinfected, were treated in a
like manner. They were placed one layer deep in sprouting boxes and
stored in a well-ventilated house for 3–4 days. After this period they
were stored in a clamp, each lot being covered with a thick layer of
straw before "earthing up". The tubers were removed from the clamp

Table VI. *The time of digging and incidence of skin spot,*
1937–8 experiment

Date of digging and disinfection with 0·5 % organo-mercurial C			Total no. of tubers	Severe skin spot %	Slight skin spot %	Clean tubers %	Commercially clean %	Total skin spot %
5 Aug. 1937	Untreated	a	146	48·6	28·1	23·3	51·4	76·7
		b	158	43·7	29·7	26·6	56·3	73·4
		Mean	152	46·2	28·9	24·9	53·8	75·1
	Treated	a	158	0·0	4·4	95·6	100·0	4·4
		b	195	0·0	4·1	95·9	100·0	4·1
		Mean	176	0·0	4·3	95·7	100·0	4·3
26 Aug. 1937	Untreated	a	230	63·9	24·8	11·3	36·1	88·7
		b	199	53·8	27·1	19·1	46·2	80·9
		Mean	214	58·8	26·0	15·2	41·2	84·8
	Treated	a	220	0·0	2·3	97·7	100·0	2·3
		b	224	0·0	8·5	91·5	100·0	8·5
		Mean	222	0·0	5·4	94·6	100·0	5·4
1 Oct. 1937	Untreated	a	162	65·4	23·5	11·1	34·6	88·9
		b	197	54·8	30·5	14·7	45·2	85·3
		Mean	179	60·1	27·0	12·9	39·9	87·1
	Treated	a	172	0·0	9·3	90·7	100·0	9·3
		b	209	1·0	3·8	95·2	99·0	4·8
		Mean	190	0·5	6·6	92·9	99·5	7·1
1 Nov. 1937	Untreated	a	238	58·4	18·9	22·7	41·6	77·3
		b	217	62·7	21·7	15·6	37·3	84·4
		Mean	227	60·6	20·3	19·1	39·4	80·9
	Treated	a	189	0·0	9·0	91·0	100·0	9·0
		b	216	0·0	4·6	95·4	100·0	4·6
		Mean	202	0·0	6·8	93·2	100·0	6·8
29 Nov. 1937	Untreated	a	183	45·9	31·1	23·0	54·1	77·0
		b	175	45·7	26·3	28·0	54·3	72·0
		Mean	179	45·8	28·7	25·5	54·2	74·5
	Treated	a	183	1·6	7·7	90·7	98·4	9·3
		b	194	1·5	9·8	88·7	98·5	11·3
		Mean	188	1·5	8·8	89·7	98·5	10·3

the following May and the method of examination and classification for
skin spot adopted was similar to that employed in 1936 and 1937. The
results, which are given in Table VI. show that severe skin spot developed
in all the non-disinfected lots of tubers, and that there was little or no
difference in the incidence of the disease irrespective of the time of digging.
For all the diggings, disinfection immediately after lifting gave excellent
control of skin spot.

V. DISCUSSION

General. O. pustulans is a soil-borne fungus and is generally assumed to be present in all soils which are suitable for its maintenance. Scottish records (Scottish Journal, 1932) show that skin spot occurred in tubers produced by non-affected seed planted in land which had been in grass for at least 20 years before cropping, and which had received no farmyard manure. This and similar evidence would suggest that in such cases the amount of infection present on the seed at planting or the disinfection of the seed prior to planting would have little, if any, effect upon the disease in the resultant crop. Nevertheless, the results of this investigation indicate that, at least in some soils and under the experimental conditions obtaining, the disinfection of affected tubers before planting does give a certain measure of control. At the same time a larger reduction in the incidence of the disease than that obtained by disinfection immediately before planting is highly desirable. The prevention of the development of the disease during storage irrespective of the conditions or the amount of infection present in the soil in which the tubers are grown is the ideal objective, and this was more nearly attained by disinfection of the tubers immediately after digging. Although the experiments dealing with disinfection immediately after digging are limited in their range in so far as soil type, potato variety and climatic conditions are concerned, yet the results obtained have been very encouraging and suggest that this method is worthy of a wider investigation.

Method of disease assessment. The incidence of the disease in all tuber samples was assessed on the presence or absence of skin-spot lesions on the tubers. The subsequent behaviour of the tubers with regard to sprouting and plant establishment in the field was not closely studied. This was chiefly due to the fact that seed tubers of the varieties used— Sharpe's Express, Kerr's Pink and Arran Banner—may be severely affected with skin spot without subsequent damage to sprouting or establishment. Various samples of tubers of all three varieties severely affected with the disease and retained for planting gave rise to healthy vigorous sprouts and excellent plant establishment. It would, probably, have been more satisfactory to have used such varieties as King Edward and Majestic in which non-sprouting and poor establishment due to skin spot have been frequently recorded, and to have assessed the incidence of the disease under three heads, i.e. tuber lesions, sprouting, and establishment; but it was not possible to obtain suitable supplies of these varieties heavily infected with the disease. On the whole, however, it

would appear justifiable to assume that the method of assessment adopted gives a true index of the incidence of the disease, and that methods of control giving satisfactory results here would be almost if not equally successful with the more susceptible varieties.

Relative values of fungicides. The most satisfactory method of treatment was found to consist of the "instantaneous" dipping (0·5–1·0 min.) of the tubers in a solution of an organo-mercury compound. The three fungicides of this type (A, B and C) used in the present work were of a proprietary nature, and particularly good results were obtained by the use of organo-mercurial C in aqueous solution at a strength of 0·5 %. Promising results were also obtained by steeping the tubers for 90 min. in a 0·1 % solution of mercuric chloride, but the tediousness of this method proved to be a disadvantage. The proprietary dust fungicide used in 1937 proved to be quite ineffective under the experimental conditions obtaining. The use of formalin was not encouraging, and in most cases little or no control of the disease was secured by this method.

The action of fungicides relative to the time of infection and the development of the disease. If it be assumed that formalin is lethal for all latent infection present on the tuber or in the soil adhering to the tuber at the time of digging, then the reason for its ineffectiveness may be found to lie in the time of infection of the tuber and the subsequent rate of development of the disease, problems which have not yet been fully elucidated. It is possible that infection occurs before the normal time of digging, a conception which is rather favoured by results obtained from the time of digging experiment 1937–8, and that the fungus remains latent in the lenticels and periderm region for some time before undergoing further development. Considerable evidence is available which indicates that in the case of diseases of ripening and stored fruit there may be a pronounced lapse of time between infection and the development of disease (Baker, 1938). If such be the case the killing of an infective principle present on or near the surface of the tuber at digging time would not ensure disease control. On the other hand, preparations of mercury used as fungicides did secure definite control, and this may be explained by their action within the lenticels and periderm region thereby killing the parasite resting there before its further development. Experiments carried out in 1937–8 showed clearly that even disinfection with organo-mercurial C gave only partial control when carried out at a date later than about 8 weeks after the digging of the crop, thus suggesting that this control measure is non-effective when the parasite has developed beyond a certain stage. Evidence that fungicidal action within the peri-

derm region is possible when organo-mercury compounds are used is
provided by the fact, that when slightly immature tubers are dipped or
if the fungicide is too strong, then the skin of the tuber may be killed and
slough off leaving the exposed tissue beneath distinctly browned. In the
case of very young tubers this may result in loss of water by the tuber and
its becoming soft and india-rubber like. A further advantage which may
accrue from the use of mercury as a fungicide is the fact that the material
dries and persists as a coating on the surface of the tuber thereby pre-
venting infection from extraneous sources during storage, whereas the
more temporary effect of formalin does not provide such protective
action.

Digging time and the incidence of skin spot. The earlier digging of
potato crops intended for use as "seed" is a practice which is probably
deserving of more attention than it has received in the past. It appears
to be making headway in Holland where it is recommended in particular
as an aid towards keeping potato stocks freer from virus infection. The
better weather conditions which normally obtain in the early autumn
with the consequent lifting of cleaner and more presentable tubers is also
a point worthy of consideration by the "seed" grower. In 1937–8 an
attempt was made to determine the effect of the time of digging upon the
incidence of skin spot in the resultant crop. The results obtained show
that the disease developed in all samples irrespective of the earliness of
digging, although tuber disinfection on the same day as digging gave
excellent control in every case. It must, however, be pointed out that
these results hold for only one variety in one year under one set of soil
conditions and, as has already been stressed, when the variability of these
three factors is taken into account, too much emphasis cannot be laid
upon these findings as general principles. Although no skin-spot lesions
were observed on any of the tubers at any of the digging times during the
experiment, yet in a previous season a very slight attack of the disease
was observed at the time of lifting a crop of the same variety.

Optimum time for disinfection. All things considered, the optimum
time for tuber disinfection for skin-spot control is as soon as possible after
digging. Although the results of 1937–8 indicate that treatment need not
necessarily be carried out immediately, they also show that when carried
out at an interval of more than 8 weeks after digging its effectiveness is
substantially decreased. The selection and disinfection of "seed" at
digging time serves a dual purpose in that it secures the prevention of
the development of skin spot during storage and ensures the planting
of comparatively disease-free seed. Further, digging time is also the

optimum time for disinfection when tuber diseases such as common scab (Cairns *et al.* 1936) and blight (Greeves, 1937) are taken into consideration. Some evidence has also been obtained and is here recorded of disinfected tubers proving to be less susceptible to storage diseases. Lastly, as the same technique is applicable in all cases, it would appear to be possible by the same treatment, i.e. disinfection of the seed tubers at the time of digging, to control not only skin spot but a group of diseases of the seed tuber, many of which are transmissible to the ensuing crop.

VI. Summary

1. Experiments carried out during 1933–8 with the object of investigating the control of skin spot (*Oospora pustulans* Owen & Wakef.) by seed-tuber disinfection are described.

2. Compounds of mercury were effective when used in solution as steeps. Formalin and a proprietary dusting compound proved to be non-effective. Proprietary organo-mercury compounds gave the most promising results, and were satisfactory when used as "instantaneous" dips (0·5–1·0 min.); mercuric chloride solution was efficient but the 90 min. dipping period employed made the method too tedious.

3. The time at which the treatment is carried out is of great importance in determining the measure of control obtained. Disinfection of the seed at the time of digging was far more effective than immediately before planting. If too long an interval (more than 8 weeks) is allowed to elapse between digging and disinfection, the value of the treatment is substantially reduced.

4. A study of the time of digging the crop upon the incidence of the disease, made during one year only, indicated that for that year early digging did not reduce the amount of the disease which developed during subsequent storage. Disinfection carried out at each of the five diggings gave satisfactory control in each case.

5. The method of treatment recommended is the same as that previously suggested for the control of common scab (*Actinomyces scabies* (Thaxt.) Güss.) and blight (*Phytophthora infestans* (Mont.) de Bary). Some evidence was obtained of its also being effective against storage diseases. It would, therefore, appear that disinfection of seed tubers at the time of digging may prove effective against a group of tuber diseases many of which are transmissible to the resultant crop.

suitable for the work; and to William Wilson for his assistance with the routine work involved. They thank the Directors and Trustees of the Northern Ireland Agricultural Research Institute for the facilities provided. Acknowledgement is also made to Messrs Bayer Products, Ltd., of London, for the grant of a research scholarship in plant pathology which has assisted materially towards the progress of the investigation.

REFERENCES

BAKER, R. E. D. (1938). Studies in the pathogenicity of tropical fungi. II. The occurrence of latent infections in developing fruit. *Ann. Bot., Lond.,* **2**, N.S. 919–31.

CAIRNS, H., GREEVES, T. N. & MUSKETT, A. E. (1936). The control of common scab (*Actinomyces scabies* (Thaxt.) Güss.) of the potato by tuber disinfection. *Ann. appl. Biol.* **23**, 718–42.

GREEVES, T. N. (1937). The control of blight (*Phytophthora infestans*) in seed potatoes by tuber disinfection. *Ann. appl. Biol.* **24**, 26–32.

MINISTRY OF AGRICULTURE AND FISHERIES (1937). Skin spot and silver scurf of potatoes. *Adv. Leafl. Minist. Agric. Fish., Lond.,* no. 279.

SCOTTISH JOURNAL (1932). Skin spot and blindness in seed potatoes. *Scot. J. Agric.* **15**, 191–6.

EXPLANATION OF PLATE XXIV

The photographs show representative portions of the crops of the variety Sharpe's Express (1935–6 experiments). In each case the crop sample is graded into three classes, viz.: A, severely affected; B, slightly affected; and C, clean tubers. The photographs were taken in June 1936.

Fig. 1. Crop grown from *non*-disinfected seed; resultant crop *not* disinfected after digging.

Fig. 2. Crop grown from seed disinfected before planting (1·0 % organo-mercurial C); resultant crop *not* disinfected after digging.

Fig. 3. Crop grown from *non*-disinfected seed; resultant crop disinfected immediately after digging (1·0 % organo-mercurial C).

Fig. 4. Crop grown from *non*-disinfected seed; resultant crop disinfected immediately after digging (1·5 % organo-mercurial B).
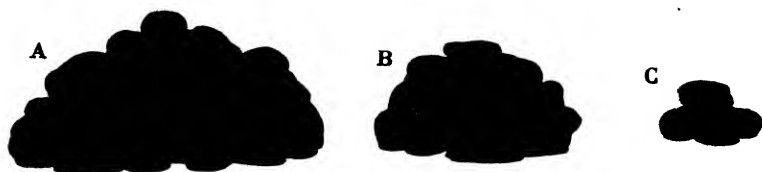
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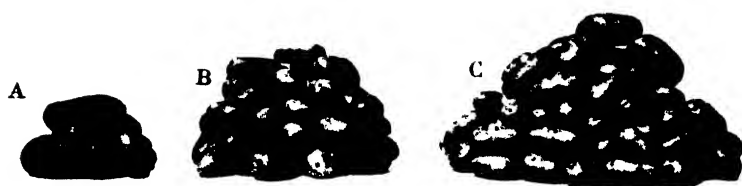
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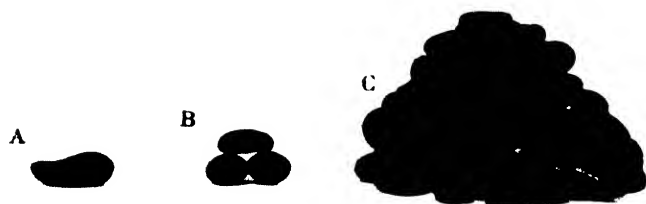
Fig. 3.

Fig. 4.

GREEVES and MUSKETT. —Skin Spot (*Oospora pustulans* Owen & Wakef.) OF THE POTATO, AND ITS CONTROL BY TUBER DISINFECTION (pp. 481–496)

SUCCESSION OF FUNGI DECOMPOSING WHEAT STRAW IN DIFFERENT SOILS, WITH SPECIAL REFERENCE TO *FUSARIUM CULMORUM*

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(With 2 Text-figures)

CONTENTS

I. INTRODUCTION

IT has long been known that a succession of fungi develops in the underground parts of a plant after infection by a primary parasite. This succession was studied by Hildebrand (1934) for strawberry root rot, by Koch (1935) for tobacco root rot, and further investigated by Hildebrand & Koch (1936) by means of direct microscopical examination of infected roots. The precise extent to which secondary parasites increase the damage done by the primary parasite is uncertain, but it was found by Hansen (1929) that pink-root infection of onions by *Phoma terrestris* was aggravated by simultaneous inoculation with *Fusarium malli*, a secondary parasite rather regularly following *Phoma terrestris* in natural pink-root infection. Again, Geach (1933) found that whereas *Urocystis tritici* alone caused 3·6 % seedling blight in wheat seedlings, and *Fusarium culmorum* 1·8 % under the particular field conditions of his experiments and with his particular isolations of these fungi, yet where the two fungi were used together a mortality of 37·8 % of seedlings was the result. Attention was first drawn by Fawcett (1931) to the fact that mixed infections of fruit and other above-ground parts of plants are more common than was formerly supposed.

Experience in this laboratory has shown repeatedly that it is difficult to isolate *Ophiobolus graminis* from the stem bases of wheat plants thoroughly infected with this fungus, and heavily encrusted with typical plate mycelium. Attention was attracted to the frequent occurrence of *Fusarium culmorum* colonies, which occasionally constituted as much as 90 % of those developing from the surface-sterilized stem bases plated out on acidified potato-dextrose agar. The results obtained from plating out *Ophiobolus*-infected stem bases of wheat and barley plants collected from seven different crops in the 1937 season are given in Table I.

Table I. *Fungi isolated from surface-sterilized stem bases of wheat and barley showing typical plate mycelium of* Ophiobolus graminis

			No. of straws plated out	% straws yielding colonies of *O. graminis*	% straws yielding colonies of *F. culmorum*
Wheat,	from	Reading, Berks	20	35	15
,,	,,	Harnhill, Wilts	92	4	38
,,	,,	Castle Ashby, Northants	36	3	6
,,	,,	Wrington, Somerset	12	0	67
Barley,	from	Fulbourn, Cambs	40	5	23
,,	,,	Tunstead, Norfolk	16	0	63
,,	,,	Bridgham, Norfolk	44	0	34

Taking into consideration the well-established reputation of *Ophiobolus graminis* as a vigorous primary parasite, and also the fact that the diseased plants showed external mycelium of this fungus alone, it is difficult to resist the conclusion that *Fusarium culmorum* was here present in the capacity of a secondary parasite. At the same time there is no intention of calling in question either the capacity of *F. culmorum* for causing a severe seedling blight of cereals (Simmonds, 1928; Bennett, 1928; Blair, 1937), or the fact that it is often the only fungus associated with the whiteheads condition in England (Bennett, 1928; Russell, 1932), Canada (Simmonds, 1928; Greaney & Machacek, 1935), the U.S.A. (Tu, 1929), Australia (Geach, 1933) and elsewhere.

Further light was thrown upon the association of *F. culmorum* with the wheat plant by the observations of Samuel & Greaney (1937), who isolated the fungus from surface-sterilized root and crown pieces taken from the bases of normal wheat plants showing no disease symptoms. A survey was made from three fields in different localities, and samples taken at intervals of about a fortnight from heading until after harvest. The percentage of *F. culmorum* isolations obtained from two out of the three fields increased in the later samplings, and was greatest in that made from the stubble after harvest. Tests on the wheat seedlings in the

glasshouse showed that the pathogenicity of these isolates of *F. culmorum* was represented by an average disease rating (Greaney & Machacek, 1935) of 21 % for thirteen isolates of the fungus. Samuel & Greaney call attention to the earlier work of Broadfoot (1934), who obtained 20–60 % of *F. culmorum* in many thousands of isolations from wheat plants taken at random from a series of rotation plots. In conclusion, they comment upon their work as follows: "It is evident that the fungus must have been present in the soil from which these healthy wheat plants were taken, exerting no appreciable parasitic effect and entering the root systems, along with other fungi only as the roots began to lose vitality after the flowering of the crop. There was then a still further development of the fungus on the stubble, after the crop had been cut. This is in contrast to fairly numerous cases reported, especially from the north of England, of appreciable damage to wheat by the fungus *F. culmorum*."

These observations, taken in conjunction, suggested that if *F. culmorum* behaved as a regular secondary parasite of plants infected by *Ophiobolus graminis*, and as a weak primary parasite of normally senescent plants, it might also be able to colonize the dead roots and stem bases of wheat stubble left in the ground after harvest. It seemed possible that this fungus might also play an important part in the normal decomposition of threshed wheat straw when buried in the soil. An investigation into the succession of soil fungi colonizing such buried wheat straw has therefore been made, and forms the subject of the present communication.

II. Methods

Wheat straw for use in these experiments was selected from the upper parts of healthy plants, and cut into pieces about 1 in. in length. One set of straws was used unsterilized so as to form an entirely natural substratum. A second set of straws was sterilized by autoclaving for 30 min. at 1 atm. pressure in a 2 % solution of sodium nitrate. This concentration of sodium nitrate was calculated to give 1 g. of nitrogen per 100 g. of dry straw, it having been found that 1 g. of dry straw takes up 3 c.c. of water. Thus, two different substrata were employed, one of them the natural unsterilized straw and the other sterilized straw with sufficient nitrogen added to ensure complete decomposition when buried in the soil.

The soils employed in the first experiment were as follows:

Rothamsted. Flinty clay loam, pH 6·8; collected from the field, February 1938.

Allotment. Similar soil to the above, but better supplied with organic matter as a result of allotment cultivation for more than 70 years, pH 6·8; collected from the field, February 1938.

Downham. Black fen soil from Norfolk, pH 7·6; collected from the field in July 1937, and stored in bag until February 1938.

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Compost. Glasshouse soil prepared by composting together 2 parts of grass sod and loam and 1 part of stable manure, stacked for 18 months, *p*H 6·8; this sample was taken from permanent glasshouse stock.

Woburn. Light sandy loam from the Woburn Experimental Station, *p*H 6·1; collected July 1937, and stored in bag until February 1938.

For the second experiment, fresh collections of the Rothamsted, Allotment and Woburn soils were made from the field, and the glasshouse compost was again taken from the permanent stock. In this experiment the Downham soil was replaced by one from Whitchurch:

Whitchurch. Clay loam overlying chalk subsoil, *p*H 8·1; collected July 1937, and stored in bag until May 1938.

The experiments were set up as follows: The soils were not air-dried, but were passed through a ¼ in. sieve to remove large lumps. Four-inch pots were used as containers: twenty-five straws were buried in each pot in five layers of five each in the different soils contained in the pots. After filling, the pots were well watered and incubated in a lightly covered wooden box over a layer of moist sand. The box was housed in the laboratory and the pots of soil kept moist by occasional waterings. The laboratory temperature fluctuated between 17 and 22° C. during the period of the experiments.

A sample of two pots was taken from each soil series at weekly intervals in Exp. I and at fortnightly intervals in Exp. II; one pot of each sample contained untreated and the other the treated straw. The straws of each sample were washed free of soil over a sieve, and further cleaned by shaking in several changes of water in a 500 c.c. conical flask. The straws were surface-sterilized by shaking for a period of 45–60 sec. in 1/1000 aqueous mercuric chloride solution, followed by a washing in six changes of sterile tap water. The surface sterilized straws were plated out (five per dish) on acidified potato-dextrose agar, adjusted to *p*H 5·0, and the plates incubated at 25° C. for a fortnight before examination of the colonies.

III. Experimental

(a) *Colonization of the straws by soil fungi*

Detailed records are given, in graphical form, only of *Fusarium culmorum, Mucor* spp. and *Penicillium* spp. These types, in virtue of their abundance, appeared to be dominant organisms in the earlier or later stages of straw colonization. The occurrence of other organisms was either too infrequent or too erratic to be worth recording in detail, but the genera included *Cylindrocarpon, Stemphylium, Gliocladium, Trichoderma, Pythium, Dicarpella, Alternaria, Geomyces, Aspergillus, Pullularia* and *Sordaria*. Two or even three fungal colonies not infrequently developed from a single piece of straw. Indeed, it must be emphasized that the fungi isolated from the straws in these experiments are not necessarily those dominant in the actual decomposition of the straw, but may merely be those most easily able to develop into colonies under the particular

conditions employed. It is possible that, with different methods of surface sterilization and incubation of the straws, somewhat different results might have been obtained.

In order to simplify the presentation of the numerous data, it was decided to graph the behaviour of *Fusarium culmorum, Mucor* spp., and *Penicillium* spp., respectively, as mean percentage occurrence in the five soils of each experiment. Thus each curve in Figs. 1 and 2 represents the results from five soils, of which an average has been made for graphical presentation. The five soils of Exp. I (Fig. 1) were Rothamsted, Allotment, Woburn, Compost and Downham; fresh collections of the first four soils were made for Exp. II (Fig. 2), but Downham was replaced by Whitchurch soil. Since these experiments are concerned especially with the occurrence of *F. culmorum*, additional data concerning this fungus are given in Table II, which shows the maximum percentage of straws yielding colonies of *F. culmorum* in the five soils of each experiment.

Table II. *Maximum percentage straws yielding colonies of* F. culmorum

	Soils					
	Roth-amsted	Allot-ment	Woburn	Compost	Downham	Whit-church
Exp. I:						
Untreated straws	95	90	85	90	75	—
N-treated straws	100	100	80	75	80	—
Exp. II:						
Untreated straws	36	56	48	36	—	56
N-treated straws	40	78	40	5	—	78

The behaviour of *F. culmorum, Mucor* spp., and *Penicillium* spp. (Figs. 1, 2) may be considered in sequence.

Fusarium culmorum. Both in Exps. I and II, the percentage of straws yielding *F. culmorum* reaches a maximum in the early stages of colonization of the straws and then declines. The nitrogenous treatment of the straws does not appear appreciably to affect the height of the maximum reached by *F. culmorum*, but hastens both the attainment of the maximum, and, particularly, the decline from the maximum. Autoclaving and nitrogen treatment thus appear to hasten the replacement of *F. culmorum* by other organisms, notably *Penicillium* spp. In Exp. II, the maximum attained by *Fusarium culmorum* is lower than that reached in Exp. I. From these two experiments, therefore, it would appear that *F. culmorum* declines in amount and activity with continued incubation of the soil, either in the field or in the store.
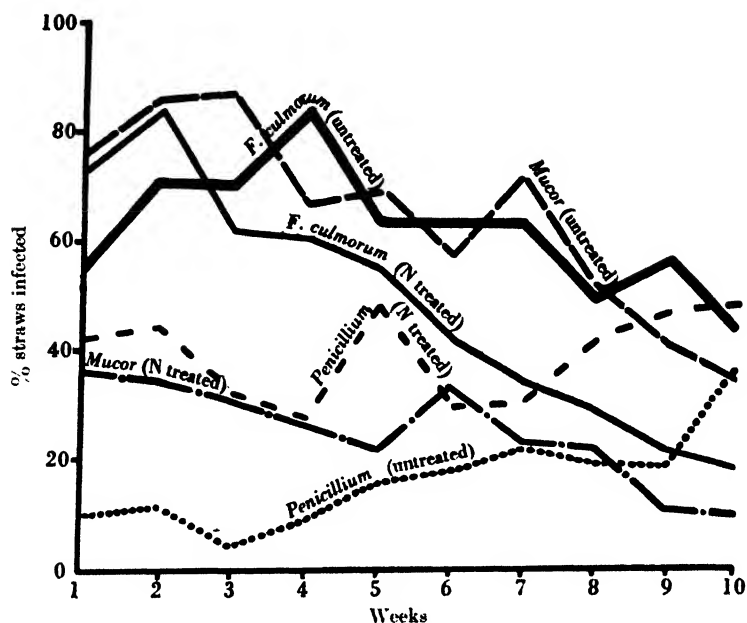
Fig. 1. Sequence of fungal development on untreated and nitrogen-treated straws buried in the soils of Exp. I.
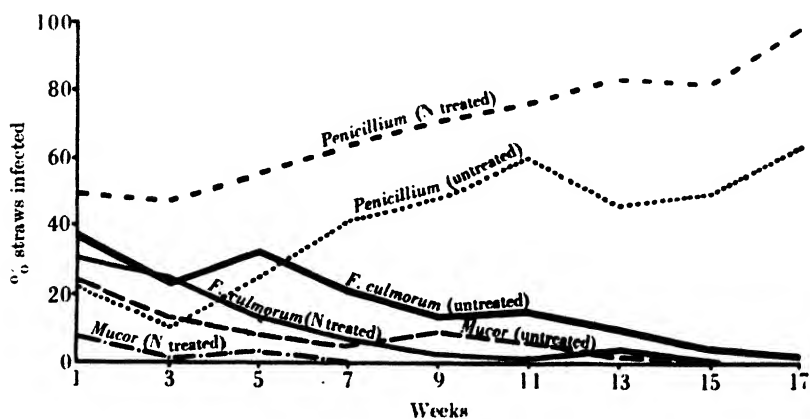
Fig. 2. Sequence of fungal development on untreated and nitrogen-treated straws buried in the soils of Exp. II.

Mucor spp. The general behaviour of *Mucor* is similar to that of *Fusarium culmorum*, inasmuch as its percentage occurrence declines from a maximum reached early in Exp. I; this initial maximum is a much lower one in Exp. II, suggesting that the activity of this fungus has also declined with incubation of the soil in field or store. The activity of *Mucor* is decidedly depressed by the nitrogen treatment of the straws in both experiments.

Penicillium spp. The behaviour of *Penicillium* is opposite to that of *Fusarium culmorum* and *Mucor*. Percentage occurrence rises from the early to the latter part of Exp. I; initial numbers are much higher in Exp. II, suggesting that the fungus has increased in relative importance with continued incubation of the soil in field or store. *Penicillium*, moreover, is decidedly encouraged by the nitrogen treatment of the straws. In all three respects just considered, therefore, the behaviour of *Penicillium* is the reverse of that displayed by *Fusarium culmorum* and *Mucor*.

General conclusions. These results emphasize the importance of *Fusarium culmorum* and *Mucor* spp. in the early stages of straw colonization by fungi, and of *Penicillium* spp. in the latter stages. The autoclaving in 2% solution of sodium nitrate has exerted a pronounced effect upon the sequence of fungal colonization, apparently by encouraging the development of *Penicillium* spp. at the expense of *Fusarium culmorum* and *Mucor* spp. The replacement of *Fusarium culmorum* and *Mucor* spp. by *Penicillium* spp. that has taken place inside the buried straws also seems to have taken place in the soils with the advancement of season from winter (February) to early summer (May), as can be seen by comparing the results of Exps. I and II respectively.

(b) *Identification and pathogenicity tests of* Fusarium culmorum *isolates*

Identification. The different isolates from Exps. I and II were compared with a type culture of *F. culmorum* originally supplied by Dr F. T. Bennett to the Imperial College of Science and Technology, and with Bennett's (1928) published description of *F. culmorum* isolates from British cereals. Included in this comparison was an isolate made in this laboratory from diseased wheat roots. The different isolates were grown on potato-dextrose agar and the cultures were 30 days old when the spore measurements were made. Ten conidia in each of the groups, 3-, 4-, and 5-septate, were measured for each isolate, and the mean lengths and breadths are given in Table III. An analysis of variance (Fisher, 1936)

made on the complete data shows that differences in spore length between the different isolates are fully significant, but that on these data, at least, it is not possible to demonstrate any connexion between spore length and pathogenicity, since the two isolates obtained in the first instance as pathogens fall well within the range covered by the saprophytic isolates.

Table III. *Mean length of ten spores of each isolate in* μ

No. of isolate ...	1	2	3	4	5	6	7	8	9	10	Type culture (Bennett)	Isolate from wheat roots
3-septate	26·3	29·4	31·7	27·4	30·3	30·7	30·1	30·4	31·6	28·3	26·5	30·7
4-septate	30·8	32·4	35·6	31·9	34·3	35·2	33·1	37·2	34·3	32·8	32·7	34·2
5-septate	34·4	35·4	39·7	33·7	39·0	37·3	34·9	41·5	37·6	36·4	34·5	37·3

Mean breadth of 10 spores of each isolate in μ

No. of isolate ...	1	2	3	4	5	6	7	8	9	10	Type culture (Bennett)	Isolate from wheat roots
3-septate	7·4	7·0	7·3	7·4	7·4	6·9	7·7	7·5	7·8	7·7	7·2	7·4
4-septate	7·9	7·2	7·5	7·6	7·2	7·1	7·4	7·3	7·8	7·8	7·5	7·2
5-septate	7·9	7·5	6·9	7·2	7·1	7·3	7·5	7·2	8·2	7·7	7·5	7·2

Pathogenicity. Pathogenicity tests to establish the identity of the fungus were done with Little Joss wheat seedlings grown in 4 in. pots of sterilized sand. The agar-disk method of inoculation employed by Garrett (1936) for *Ophiobolus graminis* was used, and selected single-spore isolates from Exps. I and II were grown on potato-dextrose agar (pH 5·0) for the provision of suitable inoculum. Five presoaked wheat seeds were planted per pot, and the pots kept outside in the open. The first test, of isolates from Exp. I, was made during the period 4–25 May (mean air temperature 10° C.), and the second test, of isolates from Exp. II, during the period 30 July to 19 August (mean air temperature 16° C.). The seedlings were washed out and the number of infected seedlings and individual roots determined by inspection in water over a white background.

The twenty isolates employed in the first test were taken in pairs from the ten consecutive samplings of Exp. I. Five isolates were taken from each of the first three samplings of Exp. II for the second test, in which was included for comparison five isolates from infected wheat roots, and the type culture of *Fusarium culmorum* (Bennett's). The results of these two pathogenicity tests are set out in Tables IV and V.

Table IV. *Pathogenicity test of* F. culmorum *isolates from Exp. I*

Isolates of F. culmorum	No. of seedlings used in test	No. killed before emergence	No. killed after emergence	% infected roots in remaining seedlings	Total % roots infected in all seedlings
1	10	4	2	60	84
2	10	2	3	66	83
3	10	5	1	66	86
4	10	5	1	70	88
5	10	3	1	60	76
6	10	3	1	77	86
7	10	5	1	82	93
8	10	2	1	56	69
9	10	5	2	70	91
10	10	7	2	100	100
11	10	3	0	63	74
12	10	3	0	45	62
13	10	5	1	82	93
14	10	6	1	85	96
15	10	6	2	85	99
16	10	4	1	52	76
17	10	5	0	43	72
18	10	5	3	95	99
19	10	6	0	36	74
20	10	7	1	36	87

Table V. *Pathogenicity test of* F. culmorum *isolates from Exp. II*

	Isolates of F. culmorum	No. of seedlings used in test	No. killed before emergence	No. killed after emergence	% infected roots in remaining seedlings	Total % roots infected in all seedlings
Isolations made after 1 week's decomposition of straws	1	10	9	0	71	97
	2	10	5	1	25	70
	3	10	3	1	47	68
	4	10	8	1	50	95
	5	10	10	0	0	100
Isolations made after 2 weeks' decomposition of straws	1	10	3	0	25	48
	2	10	7	0	57	87
	3	10	5	0	64	82
	4	10	5	1	71	88
	5	10	5	0	84	92
Isolations made after 3 weeks' decomposition of straws	1	10	1	0	8	17
	2	10	6	0	28	71
	3	10	6	1	85	96
	4	10	5	0	16	58
	5	10	4	2	64	85
Isolations from infected wheat roots	1	10	9	1	0	100
	2	10	7	3	0	100
	3	10	8	2	0	100
	4	10	8	2	0	100
	5	10	7	2	100	100
Type culture (Bennett's)	—	50	44	4	100	100

IV. Discussion

The observations reported have confirmed expectation by showing that *Fusarium culmorum* is an important primary colonizer of dead wheat straw buried in the soil. These results are a logical development of the observations of Samuel & Greaney (1937), and constitute proof of the ability of *F. culmorum* to live as a saprophyte in the soil. This fungus may now, therefore, be taken as belonging to Reinking & Manns' (1933, 1934) group of *soil inhabitants*, or *true soil fungi*. As a result of their extensive studies on the tropical soil *Fusaria*, these authors concluded that certain of the *Fusarium* spp., which occurred to an appreciable extent in all soils examined, were generally distributed in the soil, and must hence be considered as *soil-inhabiting fungi*, playing a part in the normal decomposition processes of the soil. Other of these tropical *Fusaria*, however, were isolated only locally, in past or present association with host plants. Such fungi dwindled and died out from the soil in the continued absence of suitable host plants, and were hence termed *soil invaders*. Garrett (1938) has suggested that the separation into these two ecological groups can be usefully applied to the whole group of root-infecting fungi. The *soil inhabitants* are considered to be primitive or unspecialized parasites with a wide host range, which are generally distributed throughout the soil; their parasitism is considered incidental to their saprophytic existence as members of the general soil microflora. The group of *soil invaders*, on the other hand, contains those more highly specialized parasites which are more or less closely associated with their host plants, and have little or no active saprophytic existence. Such restriction of saprophytic activity is considered to be due not so much to nutritional disabilities, as in the case of the true obligate parasites, as to the intense competition of all the saprophytic soil micro-organisms for existence upon decomposing plant remains in the soil.

These experiments have demonstrated the saprophytic capabilities of *F. culmorum*. They have further shown that this fungus is by no means confined to wheat or even to arable soils, since the soil types examined comprised two clay loams, one light sandy loam and a fen peat as arable soils, an allotment soil·devoted to vegetable growing for some 70 years, and a glasshouse compost. Whilst only six soils have been examined in these two experiments, it seems highly probable that a wider search for *F. culmorum* with the aid of the straw technique employed in this study would establish its status as a regular fungus inhabitant of cultivated soils.

V. SUMMARY

A study has been made of the sequence of fungi developing on wheat straw buried in four arable soils, an allotment soil, and a glasshouse compost. Both natural untreated straw, and straw autoclaved in a 2 % solution of sodium nitrate were employed. *Fusarium culmorum* and *Mucor* spp. appeared to be dominant organisms in the earlier stages of straw colonization, but these were replaced by *Penicillium* spp. in the latter stages of decomposition. The nitrogenous treatment of the straw favoured the development of *Penicillium* spp. at the expense of *Fusarium culmorum* and *Mucor* spp. The pathogenicity of the *Fusarium culmorum* isolates to wheat seedlings was established by inoculation experiments.

The data provided by this investigation are considered sufficient to justify the inclusion of *F. culmorum* in Reinking & Manns' (1933) group of *soil-inhabiting Fusaria*, or true soil fungi.

It is a pleasure to express my indebtedness to Mr S. D. Garrett for suggesting this problem. I am also most grateful to him for his interest in the course of the investigation. My thanks are also due to Miss M. D. Glynne for helping me in the identification of some of the genera mentioned here, and to Miss L. Cunow, for help in carrying out the experiments. An acknowledgement is finally due to Dr F. T. Bennett, who originally supplied the type culture of *Fusarium culmorum*.

REFERENCES

BENNETT, F. T. (1928). On two species of *Fusarium, Fusarium culmorum* (W. G. Sm.) Sacc. and *Fusarium avenaceum* (Fries) Sacc. as parasites of cereals. *Ann. appl. Biol.* **15**, 213.

BLAIR, I. D. (1937). An investigation on footrot of wheat in New Zealand. *N.Z. J. Sci. Tech.* **19**, 1.

BROADFOOT, W. C. (1934). Studies on foot and root rot of wheat. IV. Effect of crop rotation and cultural practice on the relative prevalence of *Helminthosporium sativum* and *Fusarium* spp. as indicated by isolations from wheat plants. *Canad. J. Res.* **10**, 115.

FAWCETT, H. S. (1931). The importance of investigations on the effects of known mixtures of organisms. *Phytopathology,* **21**, 545.

FISHER, R. A. (1936). *Statistical Methods for Research Workers.* Sixth edition. Edinburgh.

GARRETT, S. D. (1934). Factors affecting the pathogenicity of cereal foot-rot fungi. *Biol. Rev.* **9**, 351.

—— (1936). Soil conditions and the take-all disease of wheat. *Ann. appl. Biol.* **23**, 667.

—— (1938). Soil conditions and the root-infecting fungi. *Biol. Rev.* **13**, 159.

GEACH, W. L. (1933). Foot and root-rots of wheat in Australia. The influence of the combined action of *Fusarium culmorum* (W. G. Sm.) Sacc. and *Urocystis tritici* Koern. on the occurrence of seedling blight. *J. Coun. Sci. industr. Res. Aust.* **6**, 269.

GREANEY, F. J. & MACHACEK, J. E. (1935). Studies on the control of root-rot diseases of cereals caused by *Fusarium culmorum* (W. G. Sm.) Sacc. and *Helminthosporium sativum* P., K and B. II. Pathogenicity of *H. sativum* as influenced by *Cephalothecium roseum* Corda in greenhouse pot tests. *Sci. Agric.* **15**, 377.

HANSEN, H. N. (1929). Etiology of the pink-root disease of onions. *Phytopathology*, **19**, 691.

HILDEBRAND, A. A. (1934). Recent observations on strawberry root rot in the Niagara Peninsula. *Canad. J. Res.* **11**, 18.

HILDEBRAND, A. A. & KOCH, L. W. (1936). A microscopical study of infection of the roots of strawberry and tobacco seedlings by micro-organisms of the soil. *Canad. J. Res.* **14**, 11.

KOCH, L. W. (1935). Recent investigations on tobacco root rot in Canada. *Canad. J. Res.* **13**, 174.

REINKING, O. A. (1934). Parasitic and other *Fusaria* counted in Costa Rica and Panama soils. *Zbl. Bakt.* Abt. 2, **90**, 4.

—— (1935). Soil and *Fusarium* diseases. *Zbl. Bakt.* **91**, 243.

REINKING, O. A. & MANNS, M. M. (1933). Parasitic and other *Fusaria* counted in tropical soils. *Z. Parasitenk.* **6**, 23.

—— —— (1934). Parasitic and other *Fusaria* counted in Colombia soils. *Zbl. Bakt.* Abt. 2, **89**, 502.

RUSSELL, T. A. (1932). Observations on foot rot diseases of cereals. *Trans. Brit. mycol. Soc.* **16**, 253.

SAMUEL, G. & GREANEY, F. J. (1937). Some observations on the occurrence of *Fusarium culmorum* on wheat. *Trans. Brit. mycol. Soc.* **21**, 114.

SIMMONDS, P. M. (1928). Seedling blight and footrot of oats caused by *Fusarium culmorum*. *Bull. Dep. Agric. Can.* **105** (N.S.).

TU, C. (1929). Physiologic specialization in *Fusarium* spp. causing head blight of small grains. *Phytopathology*, **19**, 143.

AN ACCOUNT OF THE DEVELOPMENTAL STAGES OF SOME APHIDOPHAGOUS SYRPHIDAE (DIPT.) AND THEIR PARASITES (HYMENOPT.)

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(With Plates XXV–XXX)

CONTENTS

THE DEVELOPMENTAL STAGES OF SOME APHIDOPHAGOUS SYRPHIDAE

A GENERAL description of the larvae and pupae of Syrphidae is given by Metcalf in his paper on the Syrphidae of Maine (1916, pp. 202–5). He separates the larvae and pupae into five "types" which differ according to the food and habits of the larva, and a comprehensive key is given for the identification of these types (Metcalf, 1916, pp. 207–12, 256). In this work, Metcalf describes the distinguishing features of the aphidophagous type of Syrphid larva, which are as follows: "Body subcylindrical, flattened ventrally, and much attenuated anteriorly. Posterior respiratory process short, the spiracles either straight or convoluted, never denticulated. Interspiracular ornamentation consisting of bare hairs, nodules, ridges or lamellae, never of plumose hairs. Mouth parts of two ∧-shaped jaws. Segmental hairs single. If prolegs present, without specialized vestiture. Anterior pupal respiratory cornua apparently wanting."

The following descriptions are chiefly concerned with the pupae of additional species of aphidophagous Syrphidae which I have examined. A detailed description of the larva is not given, since the larva may be identified by the nature of the posterior respiratory trunk, which is unaltered in the pupal stage. A key has been constructed for the identification of the pupae, using my own observations, and incorporating Metcalf's descriptions of certain species. In describing the pupae, I have

used the terms employed by Metcalf; he gives a glossary of these (1916, pp. 250–61).

When known, the stage of development at which hibernation occurs in different species has been noted.

Eggs which were laid by identified Syrphidae in captivity were examined, and found to show interesting differences in the sculpturing of the chorion. Examination of the eggs of a few species only was possible, and descriptions of these are included with those of the larvae and pupae.

The sculpturing of the chorion of the eggs of Syrphidae is characteristic. The basic pattern (Metcalf, 1916, p. 202) consists of rows of elongated elevations, separated by depressed areas. These rows run more or less longitudinally, and the elevations of one row tend to alternate with those of the rows on either side. This fundamental pattern is complicated in various ways, and the ultimate pattern of the chorion is characteristic of the species, with the exception of very closely allied species, such as *Syrphus luniger* Mg. and *S. corollae* Fab., and again *S. ribesii* (L.) and *S. vitripennis* Mg. When the sculpturing of the free upper surface is complicated, it is usually different from that of the lower surface, which remains simple.

(1) and (2) *S. luniger* Mg. and *S. corollae* Fab.

I have been unable to separate the eggs, larvae and pupae of these species; separate descriptions are therefore not given.

Eggs. Although indistinguishable from one another, the eggs of these species are readily distinguished from those of other Syrphidae examined. The lower surface of the chorion is made up of relatively wide, elongated and slightly elevated areas (4–10 times as long as broad), separated by narrow, nearly parallel-sided depressions. The latter are transversed by a few short branches which connect the elevated areas (Pl. XXV, fig. 8). On the upper surface, the elevated areas are narrowed, the transverse branches of the network are more numerous, and as wide as the longitudinal ones, while the depressed areas are considerably wider. Thus, the basic undulating network is evident. The distinguishing features of the eggs of these species are the elongated "crests" on the upper surface, which coincide with the regularly alternating rows of short, narrow elevations of the chorion (Pl. XXV, figs. 6, 7).

Larva. The newly hatched larva is yellowish grey in colour, but as it grows it rapidly assumes the dark grey colour of the full-grown larva. The latter is about 1·5 cm. in length, and the transverse rows of segmental spines are prominent.

Pupa (Pl. XXV, figs. 1, 2). The dimensions of the pupa are as follows: length 7–7·3 mm. (average 7·16 mm.); width 3·6–3·9 mm. (average 3·75 mm.); height 3·0–3·4 mm. (average 3·27 mm.). A number of "starvation dwarf" pupae were formed in the laboratory, when the larvae were not given a sufficient supply of aphids, and pupated before they were fully grown. The dimensions of these pupae were: length 4·5–5·5 mm. (average 5·0 mm.), width 2·7–3·0 mm. (average 2·8 mm.), height 2·1–

2·8 mm. (average 2·37 mm.). These small pupae have not been found in nature, but they may occur when the larva is unable to find an adequate supply of food—for example, in late September and early October when aphids are scarce.

These species hibernate as pupae. The pupae are well elevated throughout their length, so that the dorsal line is convex, tapering rather abruptly to the posterior respiratory tube. Until the adult emerges, the pupa is purplish black in colour, but the colour of the empty puparium varies from a brownish black to a dark grey-black. The black colour is due partly to the pigment in the puparium, and partly to the presence of short, black integumental spines. These spines vary in size and density of distribution, being larger and more numerous in certain well-defined areas. Thus, a thin, black mid-dorsal line is evident, and immediately lateral to this are five or six pairs of oblique dark patches, which give the pupa a characteristic appearance (Pl. XXV, fig. 2). Segmental spines, so prominent in the larva, are small, and light in colour.

The posterior respiratory tube is short (about half as long as broad), dark brown in colour, and shining. The three pairs of spiracular openings are borne on well-elevated black carinae. The dorsal openings are shorter, and more elevated than the lateral and ventral pairs, which are elongated, and lie close together on the ventral side. The "buttons" or circular plates are well developed, and situated dorsally. A conspicuous, short sharp dorsal spiracular spine is developed on the median side of each spiracular plate. The interspiracular ornamentation consists of small, inconspicuous nodules (Pl. XXV, figs. 3–5).

(3) and (4) *S. ribesii* (L.) and *S. vitripennis* Mg.

The larvae and pupae of these two species are indistinguishable. The eggs of *S. ribesii* only were examined.

Eggs. The eggs of *S. ribesii* are laid singly or in groups, and while the chorion on the lower surface is simple and similar to that of the eggs of *S. luniger* described above, on the upper surface it shows a complicated sculpturing. Again the typical basic pattern is seen, but the elevations are short and broad, and end in large, shallow cup-like structures, which are very characteristic, and may be seen with the naked eye (Pl. XXV, figs. 9, 10).

Larva. The larva grows to a comparatively large size (about 2 cm.) and is a pale orange yellow colour. These species hibernate in the larval stage, pupating in early spring.

Pupa. The pupa is light brown in colour, and bare except for a few pale segmental spines. Some indication of the larval segmentation persists in the form of transverse lines, which tend to lie together in groups of three (Pl. XXVI, fig. 1). The pupa is well elevated throughout its length, the dorsal line being convex, and almost semicircular. Normal pupae are large, measuring 7·3–7·8 mm. in length (average 7·4), 3·9–4·7 mm. in width (average 4·1) and 3·5–4·2 mm. in height (average 3·85). As with *S. luniger*, a number of "starvation dwarfs" of *S. ribesii* were bred in the laboratory. These pupae measured 5·2–6·5 mm. in length (average 6·2 mm.), 3–3·7 mm. in width (average 3·1 mm.) and 2·9–3·5 mm. in height (average 3·1 mm.).

The posterior respiratory trunk is conspicuous, since it is darker in colour than the integument, and hard and shining. It is a little broader than long. The spiracles are not elevated, but are long and straight, and extend well down the sides of the tube.

The relatively wide apertures are bounded by narrow, thickened black edges. The circular plates are well developed, and have well developed dorso-ventral ridges on the median side. Conspicuous nodules, elevated above the spiracles from the interspiracular ornamentation.

(5) *S. torvus* O.-S. (Pl. XXVI, fig. 2)

The pupa of this species has been described and illustrated by Metcalf (1916, pp. 240–1, fig. 33, nos. 13–15), but the following observations may be of use in identification. In the specimen I examined, the nodules forming the interspiracular ornamentation were similar to those seen in *S. ribesii*, being well developed, and raised above the spiracles, whereas Metcalf describes them as "inconspicuous ridges". The pupa of this species is marked by transverse lines which are placed at regular intervals throughout its length. This feature serves to distinguish it from the pupae of *S. ribesii* and *S. vitripennis*, in which the transverse lines are not so well marked, and are arranged in groups of three.

(6) *S. auricollis* Mg.

Eggs and two puparia of this species were examined, but the larva was not available.

Eggs. These are laid singly, or in groups, but are not arranged in any definite manner. The sculpturing of the chorion is simple, being in the form of a fine undulating longitudinal transverse raised network. The longitudinal areas are elevated, and are joined by short transverse branches which traverse the intervening depressions (Pl. XXVI, fig. 7).

Pupa (Pl. XXVI, figs. 3, 4). The measurements of the puparia examined were: length 6·0 and 6·1 mm., width 3·2 and 3·4 mm., height 2·8 and 2·7 mm. respectively. The pupa is well elevated anteriorly, and tapers from about the middle to the posterior end. The integument is smooth and shining, with no segmental or integumental spines. The puparia examined differed considerably in colour, one being very light, while the other was deeply pigmented with black. In spite of this, the black, mid-dorsal markings were present in both specimens, and appear to be characteristic of the species. At least three anterior markings are present, and two small posterior ones visible in the deeply pigmented puparium. The anterior markings have the appearance of wide, shallow goblets, with dense black centres and bases, and sides of a lighter grey. The markings situated posteriorly are small, the central parts being black and V-shaped; while the "sides" have become separated and are situated dorso-laterally. These markings of the pupa appear to be correlated with the segmentation of the larva.

The posterior respiratory tube (Pl. XXVI, figs. 5, 6) is about as long as broad, and shows a well-marked constriction near the base. The spiracles are slightly elevated, the dorsal pair of openings more so than the lateral and ventral pairs. The lateral openings lie nearer to the ventral than to the dorsal pair. The circular plates are easily seen, and slightly elevated ridges are present on the median side. The interspiracular ornamentation consists of small, inconspicuous nodules.

(7) *S. cinctellus* (Zett)

Only one puparium of this species was examined, but it is quite distinct. The following measurements were taken: length 6·7 mm., width 3·6 mm., height 2·5 mm. The pupa is well elevated anteriorly, and the dorsal line is convex in this region

(Pl. XXVI, fig. 8). As the pupa tapers to the posterior end this line becomes concave, a feature which is accentuated by the upward turning of the ventral line posteriorly. The integument is light in colour, without markings or spines.

The posterior respiratory tube (Pl. XXVI, figs. 9, 10; Pl. XXVII, fig. 1) differs markedly from that of any other Syrphid examined. Dorsally the "wall" of the tube is about half as long as broad, but ventrally it is extremely short, almost absent. A well-defined ridge surrounds the base of the tube, forming a flattened "shelf". The dorsal spiracular openings are well elevated, but the other two pairs lie flat on the stigmal plates, close together on the ventral side. The "circular plates" are not rounded or elliptical as in other Syrphid pupae, but are rather rectangular, and elongated dorso-ventrally. On the median sides of each of these plates is a well-elevated dorso-ventral ridge, which terminates in a narrow carina. The interspiracular ornamentation consists of small, inconspicuous nodules.

(8) *S. albostriatus* (Fln.)

One larva, and one puparium of this species were seen.

Larva. The larva is rather flattened, dark grey in colour with a granulated appearance. The most striking features of the larva are the projections of the integument. These are regularly arranged, three to each segment on each side, and an elongated posterior pair. These projections increase the width, and so accentuate the flattened appearance. The projections end in well-developed spines, and the mid-dorsal pairs of segmental spines are also conspicuous.

Pupa (Pl. XXVII, figs. 2, 3). Like the larva, the pupa is dark grey in colour, and has numerous transverse indentations, set close together, which are due to the shrinking of the larval integument. The lateral projections seen in the larva are present, but the regularity of size and arrangement is lost. Unlike the larva, the pupa is not flattened, as the following measurements show. Length 7·5 mm., width 3·3 mm., height 2·5 mm.

The dorsal line is convex anteriorly, but becomes concave after the middle; the ventral line is slightly arched in front, and then turns upwards rather sharply in the posterior region. There are no integumental spines present, and segmental spines are also absent, with the exception of the mid-dorsal pairs. These are very conspicuous, for they are slightly raised, and situated in light-coloured areas, which are bounded by oblique, slightly darkened dorso-lateral bands.

The posterior respiratory tube is very conspicuous, being more than twice as long as broad, black, shining and pointed. The two spiracles are widely separated distally by a deep indentation. The great development of the stigmal plates on the median edges to form large ridges accentuates this median depression, and gives the whole structure a pointed appearance. As a result of this, the spiracles which are situated on well-elevated carinae, lie laterally, near the end of the tube, instead of in the usual terminal position. Circular plates were not distinctly seen, and the interspiracular ornamentation is restricted to small ridges which are seen when the tube is viewed from above (Pl. XXVII, figs. 4-6).

(9) *S. balteatus* (Deg.)

The larvae of this species are found abundantly on cabbage, and a large number of larvae and pupae were examined.

Larva. When fully grown, the larva is large, measuring about 2 cm. in length. It is light green (often pink-tinged) in colour, with the white dorsal lines well developed.

Pupa (Pl. XXVII, figs. 7, 8). Length 6·7–7·8 mm. (average 7·7), width 2·9–4 mm. (average 3·4), height 2·8–3·8 mm. (average 3·2). The pupa is well elevated in the anterior half, and the dorsal line is broadly convex in this region. After the middle, the pupa tapers rather suddenly, so that the dorsal line becomes concave. The ventral line is usually fairly straight. Integumental and segmental spines are absent, and the surface is smooth and shining. The colour of the puparium varies considerably, some being almost white, with hardly any markings, while others are dark brownish grey, and have deeply pigmented markings on the dorsal surface. When present, these markings are characteristic, and in the empty puparium consist of two wide curved transverse bands situated anteriorly. These markings correspond to the segmentation of the larva, as do the pairs of small, elongated dark-coloured areas which lie laterally to them. The latter are found in the posterior region also, although the larger markings are absent.

The posterior respiratory trunk is broader than long, and shows a well-marked constriction about the middle of its length. The spiracles are not elevated, and the wide apertures have narrow black edges. They are long and straight, about equidistant from one another, and extend well down the sides of the tube. The circular plates are readily distinguishable. Dorsal spiracular spines or ridges are absent, and the interspiracular ornamentation is in the form of elongated nodules (Pl. XXVII, figs. 9, 10).

Catabomba O.-S.

Puparia of the two species of *Catabomba* were examined, and found to differ strongly in the characters of the posterior respiratory trunk. This is of special interest, since the adults are very similar.

(10) *C. pyrastri* (L.) (Pl. XXVIII, fig. 5)

Larva. The larvae are green with a very distinct white dorsal stripe.

Pupa. Nine puparia of this species were examined, and found to have the following dimensions: length 7·0–8·2 mm. (average 7·9), width 3·4–4·5 mm. (average 4·4 mm.), height 3·2–4·2 mm. (average 3·7 mm.). The puparium is well elevated throughout its length, the dorsal line being almost semicircular. The integument is light brown in colour, and is covered with fine spines, which are visible only with the aid of a binocular microscope. The majority of these are light in colour, but near the posterior end they are larger, darker and more numerous than elsewhere. Small, light-coloured segmental spines are also present. The pupa is marked by transverse lines which tend to be arranged in pairs, and appear to indicate the larval segmentation.

The posterior respiratory tube is very short, being about four times as broad as long; it is dark brown in colour, and has the appearance of being sunk in the integument. A well-marked indentation separates the stigmal plates. The apertures are borne on well-elevated black, shining carinae, the dorsal pair being more elevated than the lateral and ventral pairs, which lie close together on the ventral side. The circular plates are well developed, and no dorsal-spiracular ridge or spine is present. The interspiracular ornamentation is in the form of small, but distinct black nodules (Pl. XXVIII, figs. 6–8).

(11) *C. selenitica* (Mg.) (Pl. XXVIII, fig. 1)

Two puparia of this species were examined. The puparium is very similar to that of *C. pyrastri*, but is larger, having an average length of 8·8 mm., width 5·0 mm., and height 4·7 mm. It is light brown in colour and is covered with very small, light-coloured, integumental spines. As in *C. pyrastri*, these spines are larger, darker, and more numerous posteriorly. A few light-coloured segmental spines are also present. Transverse markings of the integument are visible, but do not appear to be arranged in any definite manner.

The posterior respiratory tube (Pl. XXVIII, figs. 2–4) is short, being less than half as long as broad; it is brown and shining. The spiracular apertures are long and straight, equidistant from one another, and slightly elevated on black carinae. The dorsal apertures are shorter, and slightly more elevated than the lateral and ventral pairs. The circular plates are well developed, and a pair of conspicuous dorsal spiracular ridges are present. Minute black nodules constitute the interspiracular ornamentation.

(12) *Baccha elongata* (Fab.)

Only one puparium of this species was examined, but it is very distinctive. The pupa was formed in early spring, the species hibernating in the larval stage.

Pupa (Pl. XXVIII, figs. 9, 11). The dorsal line is broadly convex anteriorly, but it becomes concave before the middle, so that the anterior part is rather bulbous in appearance. This is accentuated by the broad flat shape of the posterior region. The ventral line follows the general shape of the dorsal one, being convex anteriorly and concave posteriorly. The pupa is elongated, the ratio of length to width and height being greater than in any Syrphid pupa of the aphidophagous type examined. Length 6·3 mm., width 2·0 mm., height 2·0 mm.

The integument is smooth and bare, both integumental and segmental spines being absent. The general colour is dark brown, but a characteristic pattern is formed by intersecting areas of a light colour. A transverse oval-shaped light-coloured area is situated mid-dorsally in the anterior region, and behind this are two pairs of wedge-shaped areas, also light in colour, but laterally placed. Extending from before the middle to the posterior end are dark-coloured areas, separated by corresponding areas of a light colour. These latter lie in the mid-dorsal, latero-dorsal, and lateral regions (Pl. XXVIII, fig. 11).

The posterior respiratory tube is brown in colour, slightly longer than broad, and situated well in front of the extremely flattened posterior edge. The stigmal plates, separated by a slight median indentation, bear the spiracular apertures which are situated on conspicuous, slightly elevated black carinae; they lie close together, but are quite distinct. The circular plates are well developed, but there is no interspiracular ornamentation, and dorsal spiracular spines or ridges are absent (Pl. XXVIII, fig. 10).

Platychirus Lep.

Eggs (Pl. XXIX, fig. 3). The eggs of *P. albimanus* (Fab.) have been examined, and found to differ from those of species of *Syrphus* in the absence of the fine raised network characteristic of the chorion of the upper surface. The ornamentation of the chorion consists of large, irregular elongated areas, which are elevated, and tend to lie in alternating rows. These are separated by narrow depressions, and a few short,

raised transverse branches traverse these, and connect the elevated areas. These areas in turn bear a large number of small circular elevations which are very numerous near the irregular projections at the edges, and give the chorion a characteristic appearance.

Larva. The larvae of species of *Platychirus* are distinguished by the purplish green colour, and the V-shaped markings on the dorsal surface. Like the puparia, the larvae do not appear to have distinguishing specific characters, but the genus is readily recognized. Hibernation occurs in the larval stage.

Pupae of *P. scutatus* (Mg.) *P. albimanus* (Fab.), *P. clypeatus* (Mg.) and *P. scambus* Staeg. have been examined, but it has not been found possible to distinguish the species.

(13) *Platychirus scutatus* (Mg.) (Pl. XXIX, fig. 1)

Pupa. The pupa is small, the measurements being: length 4·9–5·8 mm. (average 5·3 mm.), width 2·2–2·8 mm. (average 2·6 mm.) and height 2·1–2·6 mm. (average 2·3 mm.). The dorsal line is convex throughout, and the ventral line slightly so in the posterior part. The integument is light in colour, shining and without integumental or segmental spines, but is marked by a large number of transverse lines.

The posterior respiratory tube (Pl. XXIX, fig. 2) is terminal in position, and is about half as long as broad. The apertures are very small and slit-like, and the three present on each stigmal plate lie together in a black, slightly elevated "carinal plate". The circular plates are well developed; dorsal spiracular spines or ridges are absent. The interspiracular ornamentation is present in the form of minute black nodules, situated on the "carinal plates" which bear the apertures.

(14) *Sphaerophoria scripta* (L.)

The larva of this species is very common and may be found in winter, for hibernation takes place in this stage. It is 5–7 mm. long, and is yellowish green in colour, with a pair of white dorso-lateral lines, formed by the underlying fat.

Pupa (Pl. XXIX, figs. 4, 5). Fifty-two puparia were examined, and found to have the following measurements: length 5·2–6·9 mm. (average 5·7 mm.), width 2·1–3·1 mm. (average 2·6 mm.), height 2·0–3·1 mm. (average 2·5 mm.). The pupae are elongated in appearance, and the dorsal line is convex anteriorly, sloping gradually to the posterior end. The ventral line is usually straight, but may be slightly convex. The integument is generally light in colour, but some of the puparia examined were pigmented, though no characteristic colour markings were formed. Integumental and segmental spines are absent.

The posterior respiratory trunk is conspicuous, for it is about twice as long as broad, and shows a well-marked constriction about the middle of its length. The component tracheae are separated distally by a deep indentation, but they are not divergent, the sides being parallel throughout. The stigmatal apertures are wide, and are borne on conspicuous black carinae which are very slightly elevated. The circular plates are well developed, but dorsal spiracular spines or ridges, and interspiracular ornamentation are absent (Pl. XXIX, figs. 6, 7).

(15) *Sphaerophoria flavicauda* Zett. (Pl. XXIX, fig. 8)

The puparium of this species is very similar to that of *Sphaerophoria scripta*, but may be identified by its size and shape. It is small, the measurements being: length 4·5–5·2 mm. (average 4·9 mm.), width 1·9–2·4 mm. (average 2·1 mm.), height 1·8–

2·1 mm. (average 2·0 mm.). As a rule, the ventral line turns sharply upwards in the posterior two-fifths, giving the puparium a characteristic appearance.

A key has been compiled for the identification of the pupae described here, and incorporating the following aphidophagous species, described by Metcalf:

(1) *Didea fuscipes* Loew. (1911, pp. 339–40, Pl. XVI, figs. 5–7).

(2) *Syrphus torvus* O.-S. (1911, p. 342, Pl. XVII, figs. 13–15).

(3) *Paragus bicolor* (Fab.) (1911, p. 400, Pl. XIX, figs. 25–28).

(4) *Paragus tibialis* (Fln.) (1911, pp. 402–3, Pl. XIX, figs. 33–36).

(5) *Syrphus americanus* Wied. (1912, pp. 484–5, Pl. XXIII, figs. 49, 50, 55, 56).

(6) *Allograpta obliqua* (Say) (1912, pp. 536–7, Pl. XXX, figs. 68–70).

(7) *Sphaerophoria cylindrica* (Say) (1912, p. 540, Pl. XXX, figs. 72, 73, 78).

(8) *Syrphus xanthostoma* Williston. (1913, pp. 82–3, Pl. IV, figs. 84–87).

(9) *Melanostoma mellinum* (L.) (1916, p. 230, fig. 30. nos. 5, 7, 9).

(10) *Syrphus nitens* (Zett.) (1916, p. 244, fig. 34, nos. 3, 4, 6).

(11) *Pipiza pisticoides* Williston (1916, p. 226, fig. 29, nos. 2, 3).

(12) *Xanthogramma divisa* Williston (1917, p. 160, fig. 9 B, fig. 8 E, G, I, J).

(13) *Syrphus oronoensis* Metcalf (1917, p. 164, fig. 10 B, D. E, F, G).

(14) *Platychirus perpallidus* Verrall (1916, p. 170, fig. 11 H, I, J).

(15) *Syrphus knabi* Shannon (1917, p. 175, fig. 12 D, E, F, G).

Metcalf (1916) gives a key for the separation of "types" of Syrphid larvae and pupae. These various types are distinguished according to the food and habitat of the larvae, and so are designated "Filth-inhabiting type", "Boring type", "Aphidophagous type", etc. A description of the latter has been given at the beginning of the present paper.

KEY FOR THE IDENTIFICATION OF SOME SYRPHID PUPAE

1. *a.* Pupa tapering from about the middle to the posterior end (e.g. Pl. XXVII, fig. 8) **2**

   b. Pupa not tapering, the dorsal line being convex,[1] and the curve from the anterior to the posterior ends almost semicircular (e.g. Pl. XXV, fig. 1) **12**

2. *a.* Pupa tapering rather suddenly after the middle, so that the dorsal line is at first convex and then concave **3**

   b. Pupa tapering gradually towards the posterior spiracular trunk ... **7**

3. *a.* Pupa smooth, and without marginal serrations **4**

   b. Pupa not smooth, marginal serrations present **6**

4. *a.* Pupa three or more than three times as long as broad or as "high".

   b. Pupa two or two and a half times as long as broad or as "high". Usually two or three dark transverse markings on the antero-dorsal surface. Posterior spiracular trunk short, with rather straight, non-elevated spiracles (Pl. XXVII, figs. 7–10). **Syrphus balteatus** (Deg.).

5. *a.* Pupa with short posterior spiracular trunk; spiracles arranged close together and not elevated. The markings on the antero-dorsal surface are characteristic (Pl. XXVIII, figs. 9–11). **Baccha elongata** (Fab.).

[1] In *Syrphus auricollis* Mg. the pupa is only slightly convex, and may be confused with (*a*).

5. *b.* Pupa with the posterior spiracular trunk elongated, and having a characteristic constriction two-thirds the distance from the base. Slit-like spiracles, moderately elevated and short. Prominent dorsal-spiracular ridge, rather crescent-shaped. Interspiracular ornamentation consists of ridges bearing single small whitish setae.

Syrphus oronoensis Metcalf.

6. *a.* Pupa with marginal serrations arranged in groups of three. Six or seven prominent transverse blackish bands mark the integument. Posterior end truncated. Posterior spiracular trunk elongated with the stigmatal plates separated by a shallow depression. Dorsal spiracular spine inconspicuous, but interspiracular ridges broadly carinate and rugose. . **Xanthogramma divisa** Will.

   b. Pupa with marginal serrations not regularly arranged in groups of three. No definite colour markings, but with prominent dorsal setae. Posterior end elongated. Posterior spiracular trunk elongated, appearing to be pointed. Dorsal spiracular ridge very well developed, and the apertures lie on the sides of the tube (Pl. XXVII, figs. 2–6).

Syrphus albostriatus (Fln.)

7. *a.* Pupa smooth **8**
   b. Pupa not smooth, but with well-developed segmental spines. No marginal serrations. Posterior spiracular trunk elongated. Dorsal spine well marked. **Paragus tibialis** (Fln.)

8. *a.* Posterior respiratory trunk very short—less than half as long as broad   **9**
   b. Posterior respiratory trunk elongated—at least twice as long as broad   **10**

9. *a.* Posterior respiratory trunk of usual form, with flattened distal surface. Length of tube equal all round. No dorsal spiracular ridge or spine.

Melanostoma mellinum (L.)

   b. Posterior respiratory trunk much longer anteriorly than posteriorly, and having a ridge around the base. Spiracular plates elongated and parallel sided. Dorsal spiracular ridge well developed, carinate. Dorsal spiracles well elevated and separated from the others which lie flat (Pl. XXVI, figs. 8–10 and Pl. XXVII, fig. 1). **Syrphus cinctellus** (Zett.)

10. *a.* Posterior spiracular trunk with short but distinct spur-like nodule on each interspiracular space, which is continued down the side of the tube as a more or less evident ridge. **Allograpta obliqua** (Say).
    b. Posterior spiracular trunk without interspiracular spines **11**

Sphaerophoria sp.

11. *a.* Pupa small (average length 4·9 mm.) with characteristic elevation of the postero-ventral line (Pl. XXIX, fig. 8).

Sphaerophoria flavicauda Zett.

    b. Pupa larger (average length 5·8 mm.) without marked elevation of the postero-ventral line (Pl. XXIX, figs. 4–7).

Sphaerophoria scripta (L.) and **Sphaerophoria cylindrica** (Say)

12. *a.* Pupa smooth, usually light in colour **13**
    b. Pupa spiny, usually dark in colour **19**
13. *a.* Pupa small (4·5–5·5 mm. long, 1·5–2 mm. wide, 1·5–2 mm. high). Posterior spiracular trunk short with the spiracles slit-like and situated on a black, slightly elevated "plate". No interspiracular spines or ridges (Pl. XXIX, figs. 1, 2). **Platychirus** spp.
    b. Pupa larger in all dimensions. Posterior respiratory trunk long, but not particularly so; the spiracles well developed, separate. Interspiracular ornamentation present, and also dorsal spine or ridge **14**
14. *a.* Posterior, slit-like spiracles greatly elongated and very sinuous, the dorsal one much elevated. Interspiracular ornamentation consists of inconspicuous nodules. **Syrphus nitens** (Zett.)
    b. Posterior spiracles straight or curved, but never sinuous **15**
15. *a.* Pupa with characteristic colour markings. Dorsal line not broadly convex. Interspiracular ornamentation in the form of small, carinate knobs (Pl. XXVI, figs. 3–6). **Syrphus auricollis** Mg.
    b. Pupa without definite markings. Dorsal line almost semicircular. Interspiracular ornamentation consists of well-elevated ridges or nodules ... **16**
16. *a.* Posterior respiratory tube as long, or slightly longer than broad ... **17**
    b. Posterior respiratory tube about one-third broader than long ... **18**
17. *a.* Puparium exceptionally inflated dorsally, the posterior inflation being equal to, or greater than that anteriorly. Interspiracular spines short, blunt, spur-like, but prominent, standing well above the spiracular openings. Dorsal spiracular spine short. **Syrphus xanthostoma** Will.
    b. Puparium not exceptionally inflated. Interspiracular ornamentation is of short, sharp, well-elevated carinae. Well-developed dorsal ridge.
    Syrphus knabi Shannon
18. *a.* Pupa with transverse markings arranged at regular intervals, not in groups (Pl. XXVI, fig. 2). **Syrphus torvus** O.-S.
    b. Pupa with transverse markings light, irregular, with a distinct tendency to be arranged in groups of three or four, apparently marking the larval segmentation (Pl. XXVI, fig. 1).
    Syrphus ribesii (L.) and **S. vitripennis** Mg.
19. *a.* "Segmental and integumental spines not visible to the naked eye, but give the pupa a 'spinulose' appearance when seen under the lower power of the microscope" (Metcalf). Spines light in colour. General colour a light testaceous brown **20**
    b. Segmental spines plainly visible; light or dark in colour. Integumental spines are short, sharp and black in colour when present **22**
20. *a.* Pupa small (4–5 mm. long); the posterior spiracular process elongated (about twice as long as broad), and having a definite constriction about the middle. Interspiracular spaces with long, slender hairs.
    Pipiza pisticoides Will.

b. Pupa large (7–9 mm. long). Posterior spiracular trunk one-third or less than one-third as long as broad. Interspiracular spaces with small, inconspicuous nodules **21**

Catabomba spp.

21. a. Posterior spiracles well elevated on broad black carinae, the dorsal spiracles more elevated than the others. Dorsal spiracular spine or ridge absent (Pl. XXVIII, figs. 5–8).   **Catabomba pyrastri** (L.)

b. Posterior spiracles narrow, slightly elevated. Dorsal spiracular ridge present (Pl. XXVIII, figs. 1–4).   **Catabomba selenitica** (Mg.)

22. a. Integumental spines present, black **23**

b. Integumental spines absent. Segmental spines present, and arranged in a definite order. Posterior spiracular trunk very slightly longer than broad. Interspiracular spaces with no visible spines, but the dorsal spiracular ridge is well developed.   **Paragus bicolor** (Fab.)

23. a. Segmental spines black, conspicuous, and "crown the summits of conspicuous conical elevations". Posterior respiratory tube hard, black, with elevated stigmata. Interspiracular ornamentation of thin, laminate ridges, as high as the stigmata, two to each space; they run down the side of the tube, which is short.   **Didea fuscipes** Loew.

b. Segmental spines light in colour, inconspicuous. Posterior spiracular trunk short, with well-elevated stigmata, the lateral and ventral pairs close together. Dorsal spiracular spine well developed, sharp. Interspiracular ornamentation of inconspicuous nodules **24**

24. a. Integumental spines evenly distributed.   **Syrphus americanus** Wied.

b. Integumental spines larger and darker in patches, there being several dorso-lateral pairs of these (Pl. XXV, figs. 1–5).

Syrphus luniger Mg. and **Syrphus corollae** Fab.

SOME PARASITES OF SYRPHIDAE

Syrphidae are not subject to a very high percentage parasitism, but during the course of this work several hymenopterous parasites have been bred. In the following account, an attempt has been made to arrive at some conclusions regarding the biology of these parasites from records kept by Dr O. W. Richards of the Imperial College of Science and Technology and myself. Dr Richards has kept records of any Syrphid larvae and pupae he came across while engaged in his own work during the years 1932–6 and I am indebted to him for the use of these records. All specimens were obtained at Slough, Bucks., and all Dr Richards' were collected on cabbage, where they were feeding on *Brevicoryne brassicae* (L.) and *Myzus persicae* (Sulz.).

HYMENOPTEROUS PARASITES BRED FROM SYRPHID PUPAE

(1) ICHNEUMONOIDEA.

(a) BASSINAE.

Bassus laetatorius (Fabr.).
B. annulatus Grav.
Homocidus signatus (Grav.).
H. tarsatorius (Panz.).
H. nigritarsis (Grav.).
H. pictus (Grav.).
Promethus monticola Sey.
P. sulcator (Grav.).

(b) CRYPTINAE.

Gelis sp.

(2) CHALCIDOIDEA.

(a) ENCYRTIDAE.

Syrphophagus aeruginosus (Dalm.).

(b) PTEROMALIDAE.

Pachyneuron formosum Walker.

(3) PROCTOTRUPOIDEA (Calliceratidae).

Lygocerus sp.
Trichosteresis försteri Kieff.
Conostigmus sp.

(1) *Bassus*

With the exception of 1 ♂ *Bassus annulatus* Gr. which was bred from the puparium of *Syrphus luniger*, only ♀♀ of *Bassus laetatorius* (Fab.) have been bred.

A pupa of *Syrphus luniger* was collected on 11 September 1933, and on 2 February 1934 a ♂ *Bassus annulatus* emerged. This is early in the year for the emergence of the parasite, but the puparium was kept indoors during the winter. Twenty-six ♀♀ of *B. laetatorius* were bred, the hosts being *Syrphus luniger*, *S. balteatus* and *Sphaerophoria scripta*.

The records show that there are at least two generations of this parasite per annum, and there is some indication of the existence of one or two more. The parasite hibernates in the body of the larva of *Sph. scripta* and *Syrphus balteatus*; for these Syrphidae do not pupate until the

spring, while it is a parasite of the puparium of *S. luniger* during the winter, since hibernation occurs in the pupal stage in that species.

The first generation of *Bassus laetatorius* emerges in spring (March–May) and the last in the autumn (September and October). This is clearly shown by the records kept. Little or no collecting was done during July and August, except in 1935, but the records for that year show that individuals of this species emerge during these months, and suggest that at least one generation of the parasite emerges about this time. Morley (1911) says that this Ichneumonid "is abundant from the end of July to the beginning of September", an observation which supports the suggestion that one or more generations emerge at this time of the year.

Three females which emerged in May 1935 were put in tubes containing eggs of *Syrphus luniger*, *S. ribesii* and *Melanostoma mellinum* respectively. A cut and moistened raisin was put in for food. In each case the ♀♀ pierced the Syrphid eggs present, and a ♀ *Bassus laetatorius* emerged from one of the pupae of *Melanostoma mellinum*, which developed from these eggs. The duration of the pre-adult period was about five weeks.

Again, a ♀ *Bassus laetatorius* was put in a tube containing eggs and first-stage larvae of *Syrphus luniger*. The parasite on this occasion proceeded to eat the eggs, using the young larvae for oviposition. Later, a ♀ *Bassus laetatorius* emerged from a puparium which developed from one of these larvae. Parthenogenesis is the rule in this species, and oviposition occurs soon after the emergence of the adult parasite. The parasite will live for a considerable time in tubes containing a cut, wet raisin. In the laboratory, under these conditions, I have found the parasite to live as long as three weeks.

According to the records available, *Syrphus balteatus* and *Sphaerophoria scripta* are the chief hosts of this parasite at Slough, but it has been bred from pupae of *Syrphus luniger*, *Platychirus* sp. and *Melanostoma mellinum*, and has been observed to attack the eggs of *Syrphus ribesii*. In this instance, the records may be misleading, for larvae and pupae of *S. balteatus* and *Sphaerophoria scripta* are found in far greater numbers than the others on cabbages, where most of the collecting was done.

The percentage parasitism of *Syrphus balteatus* by this Ichneumonid was 3·0, and 3·6 % of the larvae and pupae of *Sphaerophoria scripta* were attacked. An account of the biology of *Bassus laetatorius* is given by Kelly (1914).

(2) *Homocidus*

Four species of this genus have been bred from Syrphid pupae, and of these *Homocidus signatus* Gr. was by far the most common, there being 22 (14 ♀♀ and 8 ♂♂) of this species out of a total of 36.

(a) *Homocidus signatus* (Grav.).

The records show that the biology of this species resembles that of *Bassus laetatorius* described above, except that ♂♂ are produced. There are at least two generations per annum; the members of the first emerge in spring and early summer, and those of the other appear during September and October. As mentioned above, little collecting was done in July and August, except in 1935, and the records for that year indicate the emergence of another generation during the latter month. It is certain that *Homocidus signatus* attacks the host before the pupal stage, for parasites emerged from puparia which had been formed in captivity.

An interesting feature is the marked preference shown by this species for selecting *Sphaerophoria scripta* and *Sph. flavicauda* as hosts. I have no records of *Homocidus signatus* parasitizing *Syrphus balteatus* or *Platychirus* sp., though other species of the genus have been bred from these Syrphidae. The following table illustrates this observation:

	No. of Syrphid larvae or pupae collected	No. parasitized by *Homocidus signatus*
Sphaerophoria scripta	350	22
Sph. flavicauda	18	3
Syrphus balteatus	399	0
Platychirus spp.	29	0

The percentage parasitism of *Sphaerophoria scripta* by *Homocidus signatus* was 6·3. The number of *Sphaerophoria flavicauda* collected was not sufficient to determine the percentage parasitism.

(b) *Homocidus tarsatorius* (Panz.).

The emergence of two adults of this species from puparia of *Syrphus balteatus* was recorded. One emerged at the end of August 1933, from a puparium which had been collected as such early in the same month, while the other emerged in April 1936, the host having been collected as a larva in September 1935. These records again show the occurrence of at least two generations per annum, characteristic of these Ichneumonid parasites.

(c) *Homocidus pictus* (Grav.).

One ♀ was bred from the puparium of *Platychirus* sp. in June 1934. The host was collected at the larval stage in October 1933.

All these observations show that the biology of the various species of *Homocidus* is very similar to that of *Bassus* spp. This is not surprising among members of the same group which attack the same hosts. Thus there are at least two generations of the parasite during the year, and the host is attacked before the pupal stage.

It is well known that *Bassus laetatorius* lays its eggs in the eggs and young larvae of the host. Dr O. W. Richards observed the ♀ of *Homocidus cinctus* (Grav.) laying in the egg of a Syrphid.

(3) *Promethus*

Two species have been bred from Syrphid puparia, namely: *Promethus monticola* Sey. and *P. sulcator* (Grav.).

(a) *Promethus monticola* Sey.

Nine individuals of this species were bred, and the biology is very similar to that of the other Bassines. Again the records show the occurrence of at least two generations per annum. Like the species of *Bassus* and *Homocidus* this parasite attacks the host before the pupal stage.

. All the *Promethus monticola* obtained were bred from puparia of *Sphaerophoria*, eight from *Syrphus scripta* and one from *S. flavicauda*.

(b) *Promethus sulcator* (Grav.).

Dr O. W. Richards bred one specimen of this parasite from the puparium of *Melanostoma mellinum*. This was collected in the larval stage, on dock at Slough, Bucks.

In his description of this species, Morley (1911, p. 118) says, "Holmgren once bred it from an undetermined species of *Syrphus*, in Sweden", which is the only hint we have respecting its economy.

(4) *Gelis* sp.

One ♀ of a species of *Gelis* was bred from the puparium of *Melanostoma mellinum*. This puparium ·was collected on 18 August 1932 on *Alliaria officinalis* Andrz., under willow, and on 19 August a long narrow parasitic egg was noticed through the puparial skin, near the head of the pupa. On 20 August the larva of the parasite was visible through the skin, and

the adult emerged on 8 September. Thus, the only detail available regarding the biology of this parasite is that the pre-adult period lasts for about 17 days.

MOUTH PARTS OF THE MATURE LARVA OF SOME ICHNEUMONID PARASITES

(Pl. XXX, figs. 1–4)

The head of the last larval stage of some of the Bassinae was examined. In order to do this, the puparia from which the parasites had emerged were soaked overnight in a solution of caustic soda, then dissected, and the cast larval skins they contained were mounted in Faure's fluid, without staining. This method was used by Thorpe (1930, p. 404), and the nomenclature used here is, as far as possible, that given in his paper.

It was found that the mouth-parts of all the species examined showed a similar general plan, but differed considerably in detail, so that it was possible to separate and identify the genera, and also some of the species.

Bassus laetatorius (Fab.) (Pl. XXX, fig. 1).

The labial ring is well developed, broadly rounded, and produced to a sharp point at the closed end, while the tips of the "arms" on the open side are rounded. The labrum is present in the form of a sclerotized ridge, bearing sensillae, and lies between and slightly dorsal to the tips of the labial "arms". The mandibles are well defined, the apices being heavily sclerotized, but the basal articulatory part is not fully formed. A pair of structures situated laterally to the mandibles and the labial ring, are the mandibular struts. These are similar to the mandibular struts described by Thorpe as characteristic of the Ophionines (1930, p. 405), for they are "typically Y-shaped, with a long stem and short arms". "The upper arm is merely a condyle which articulates with the mandible, the lower arm . . . is the maxillary strut." There is no indication in this species of the labral and labial struts described by Thorpe.

Characteristic features of the larval mouth parts of the Ichneumonid parasites examined are the prominent sensillae. In this species, there are three pairs, viz., the labial sensillae situated within the labial ring, the maxillary sensillae found laterally to the arms of this labial ring, and a small pair, probably the labral sensillae, which lie near the tips of the arms and lateral to the labral ridge.

Homocidus spp.

The larval mouth-parts of four species of this genus were examined, and while those of *Homocidus tarsatorius* (Panz.) show specific characteristics, the others are indistinguishable from one another.

The presence of a pair of irregular sclerotized areas, rather like sensillae in formation, and lying latero-dorsally to the labrum, appears to be characteristic of this genus.

(a) *Homocidus signatus* (Grav.), (Pl. XXX, fig. 2). *H. nigritarsis* (Grav.) and *H. pictus* (Grav.) The labial ring, mandibles, labral ridge, labial sensillae, labral sensillae and maxillary sensillae, are all present. The labial ring is of the usual form, but instead of being rounded, it is rather angular, with two well-defined latero-ventral "corners", while the tips of the "arms" are narrow and almost pointed.

Apart from the slight indication of the sclerotization of the hypopharyngeal region, no other structures are seen in this species at this stage, the labial, maxillary and mandibular struts being absent.

(*b*) *Homocidus tarsatorius* (Panz.) (Pl. XXX, fig. 3). The mouth-parts of the last larval stage of this species are essentially the same as those of the other species of *Homocidus* described above, except that the tentorial structures are well developed and characteristic.

The labial ring, sensillae and labral ridge are very similar to those found in other species of the genus, but the tips of the arms are not attenuated to any extent. The "mandibular-maxillary" struts are almost circular, with the large, conspicuous mandibles articulated to the dorsal parts. Both labral and labial struts are developed, but they are small, and their shape is ill-defined.

Promethus monticola Sey. (Pl. XXX, fig. 4).

The arrangement of the larval mouth-parts of this species conforms to the general plan, and the essential features are easily noted. Labial ring, labral ridge, mandibles, labial and maxillary sensillae are present, but do not show any specific characteristics. The pair of sensillae designated "labral" in the descriptions given above are absent.

The special features of the mouth-parts of the last larval stage of this species are the well-developed tentorial structures. The labial, mandibular, maxillary and labral struts have fused to form a pair of structures which lie one on each side of the labial ring, and in which the component parts are distinct.

Chalcidid parasites

(1) *Syrphophagus aeruginosus* (Dalm.).

On 27 June 1935, a Syrphid larva, found later to be that of *Syrphus torvus* O.-S., was collected on *Pinus sylvestris* on Bagshot Heath, Surrey. The larva pupated on 2 July, and on 25 July, 2 ♂♂ and 5 ♀♀ of a parasite emerged. These have been identified as *Syrphophagus aeruginosus*, usually known as *Microterys aeruginosus* (Dalm.).

One ♀ died almost immediately, and of the others, two pairs and two separate ♀♀ were put in tubes containing larvae of *Syrphus luniger* Mg., *Sphaerophoria scripta* (L.) and *Melanostoma mellinum* (L.) respectively. 2 ♀♀ and 2 ♂♂ were dead on 29 July and the others died on 2 August. The Syrphid larvae used in this experiment pupated later, but no parasites emerged.

This parasite must attack the host before the pupal stage, since the parasites emerged from a puparium which had formed in captivity.

Dr A. B. Gahan, of the U.S. National Museum, confirmed the genus as *Syrphophagus*, and suggested that European Hymenopterists had probably described it under *Microterys*. It agrees perfectly with Mayr's description (1876, p. 723) of *Encyrtus aeruginosus* Dalm.

(2) *Pachyneuron formosum* Walker.

This Chalcidid has been bred frequently at Slough from pupae of *Sphaerophoris scripta* and *Syrphus balteatus*. The records show that there are at least two generations per annum, and it is possible that one or more occur during the summer. The adults of the first generation emerge in spring and early summer (March–June), having hibernated as ecto-parasitic larvae on the body of the pupa of the Syrphid host. The last generation emerges in September and early October. Breeding experiments conducted in the laboratory suggest that one or more generations occur between these two, and the apparent absence of corresponding generations in nature is probably due to the small amount of collecting done during July and August.

When a brood of *Pachyneuron formosum* emerged they were put in a 3 × 1 in. specimen tube which contained a Syrphid pupa, and also a cut and moistened raisin, fixed on a pin which was suspended from the muslin covering the open end. Several of the attempts to breed the parasite were unsuccessful, for the host was sometimes too far advanced in development to be affected by the parasite, or else the pupa was already dried up and dead. A number of the experiments were successful, and the following is an account of some of these.

On 29 May 1935, a brood of 6 ♀♀ and 1 ♂ *P. formosum* emerged, and were put in a tube containing a raisin and the pupa of *Syrphus luniger*. Females were seen to pierce the puparium with the ovipositor. but a ♂ *S. luniger* emerged on 16 June. 2 ♀♀ of this brood of parasites died on 30 May, and on 31 May the remainder were put in another tube with another pupa of *Syrphus luniger*. No parasites emerged, and when the puparium was cut open on 26 June, the pupa was found to be well advanced in development but dried up. On 3 June, these same 4 ♀♀ and 1 ♂ *Pachyneuron formosum* were still alive, and were now put in a tube with a pupa of *Syrphus ribesii*. The ♀♀ were not actually seen to pierce the puparium. and all were dead by 26 June. Two days later, 28 June, a brood of 9 ♀♀ and 4 ♂♂ of *Pachyneuron formosum* emerged from this puparium. On the same day these were sorted and put in tubes containing puparia of *Syrphus ribesii*. *S. luniger*, and *S. corollae*, which had developed from eggs laid in the laboratory. There were 2 ♀♀ and 1 ♂ *Pachyneuron* in three of the tubes. and 3 ♀♀ and 1 ♂ in the other. The ♀♀ pierced the puparia almost immediately, and all the parasites of this brood were dead by 12 July. On 22 July, 10 ♀♀ and 3 ♂♂ *P. formosum* emerged from the puparium of *Syrphus corollae*, and 2 ♀♀ from one of

S. luniger. 4 ♀♀ and 2 ♂♂ emerged from the other *S. luniger* on 25 July, while the pupa of *S. ribesii* was found to be dead when the puparium was opened on 26 July.

These parasites were now paired, and put in tubes with pupae of *S. balteatus, Sphaerophoria scripta* and *Syrphus luniger.* The ♀♀ were seen to pierce the puparia, and when a puparium of *S. luniger* was opened on 29 July, ectoparasitic larvae of the Chalcid were seen. The other puparia were opened too, but the Syrphid pupa had not withdrawn from the pupal skin, and so no eggs or larvae were seen, and the pupa was killed during the experiment.

On 3 June 1935, a brood of 4 ♀♀ and 2 ♂♂ of *Pachyneuron formosum* emerged from the puparium of *Syrphus balteatus* which had been collected in August 1934. These were put in a tube containing a pupa of *S. luniger.* All the parasites were dead on 28 June, but on 1 July a brood of 5 ♀♀ and 3 ♂♂ of the parasite emerged, and put in tubes containing pupae of *S. balteatus, Sphaerophoria scripta,* and *Syrphus corollae.* 4 ♀♀ and 1 ♂ *Pachyneuron* emerged from the puparium of *Sphaerophoria scripta* on 26 July, while the others were found to be dead.

These experiments show that the length of the pre-adult period in the laboratory is from 24 to 27 days. Laboratory conditions in summer do not differ greatly from those in the field, and so one may assume that this approximates to the length of the developmental period in nature.

The puparia of the host are attacked soon after the parasites' emergence, and the females were observed to keep the ovipositor inserted in the puparium for as long as 3 hr. The number of females in a brood always exceeded the number of males, characteristic proportions being 10 ♀♀ : 2 ♂♂, 8 ♀♀ : 3 ♂♂, 9 ♀♀ : 1 ♂, and there is one record of 20 ♀♀ : 5 ♂♂.

Earlier in the present paper it has been noted that *Sphaerophoria scripta* and *Syrphus balteatus* usually pass the winter in the larval stage, but it was also stated that *Pachyneuron formosum* hibernates as an ectoparasitic larva on the pupa of its hosts, which were the Syrphids named above. This appears to be contradictory, but the parasitized puparia were collected in August, September and early October of the previous year; if not parasitized the fly would almost certainly have emerged before the winter.

The following table shows the number of pupae collected during the months when they were to be found on cabbages, and also shows the figures on which the percentage parasitism of the puparia of *Sphaerophoria scripta* and *Syrphus balteatus* is based.

No. collected in	*Syrphus balteatus*	*Sphaerophoria scripta*
May	0	0
June	27	9
July	40	9
August	60	52
September	142	174
October	79	57
November	0	0
Total	348	301
No. parasitized	28	44
∴ % total parasitism	8	14·6
No. parasitized by *Pachyneuron*	12	12
∴ % parasitism by *Pachyneuron*	3·6	4·4

Pachyneuron formosum has been bred from the puparia of *Syrphids* by many workers. The majority of parasitized pupae recorded are those of *Syrphus balteatus*, *S. ribesii*, and (in this paper) *Sphaerophoria scripta*, but Voukassovitch (1925, p. 171) records *Syrphus vitripennis*, *S. pyrastri* (*Catabomba pyrastri*) and *Melanostoma mellinum* as hosts. I have also been able to induce the parasite to attack the pupae of *Syrphus luniger*, *S. ribesii* and *Melanostoma mellinum*, but of the Syrphids collected in nature only *Sphaerophoria scripta* and *Syrphus balteatus* were attacked. The parasite may have a preference for the puparia of these two Syrphids as hosts, but the available data is not sufficient to establish such a suggestion.

Faure (1923, pp. 255–8) has described the oviposition and development of *Pachyneuron* sp. parasitic on the pupa of *Syrphus balteatus*. Voukassovitch (1925, p. 171) records a species of *Pachyneuron* as a parasite of the Ichneumonid parasite of a Syrphid, and says that *Pachyneuron* may be a primary parasite of the Syrphid pupa, but very rarely. From the records of Dr O. W. Richards. and from my own observations, it would appear that the reverse is true at Slough, *Pachyneuron* being generally a primary parasite of the Syrphid, and occasionally a hyperparasite. Salt (1936, p. 10) agrees with this view, and Voukassovitch was perhaps dealing with another species of *Pachyneuron*. *P. formosum* is the species of this genus recorded generally as bred from Syrphid pupae.

Proctotrupid parasites (*Calliceratidae*)

(1) *Lygocerus* sp.

A brood of nine *Lygocerus* sp. emerged on 1 July from a pupa of *Sphaerophoria scripta*, the larva of which had been collected on 10 May. On examination, all these parasites were found to be ♂♂, and so no further parasitism was possible. Of these, four were dead on 13 July, and

the remainder by 17 July. The parasites were always kept in a 3 × 1 in. specimen tube which contained a moistened raisin for food, and had muslin over the open end. This method proved to be very successful.

Mr G. E. T. Nixon of the British Museum (Natural History) kindly identified the genus, but no specific name could be given, owing to the confusion into which the taxonomy of the group has fallen.

(2) *Trichosteresis försteri* Kieff.

Dr O. W. Richards's records show the emergence of two broods of this parasite from Syrphid puparia. The record of the first brood was published (Richards, 1930, pp. 277–8) and the host was described as "an unidentified Syrphid pupa". This is now found to be the puparium of *Sphaerophoria scripta*.

The other brood (2 ♀♀ and 1 ♂) emerged from the puparium of *Syrphus luniger* on 1 August 1933. The larva of this Syrphid was collected on cabbage on 23 June, and pupated on 26 June, so that the parasite must have attacked the host before the pupal stage.

(3) *Conostigmus* sp.

Four puparia of *Syrphus balteatus* were collected in August 1933, three on 22 August and one on 24 August. All contained larvae of the Proctotrupid parasite *Conostigmus* sp. Although kept indoors, these parasites hibernated within the Syrphid puparium and did not emerge until the summer of the next year—end of May until the beginning of July. This suggests that there is only one generation per annum of this parasite. All the parasites in one puparium died before emergence; of the others 9 ♀♀ and 1 ♂ emerged from one, and one from each of the other two.

Since the hosts were collected as puparia it is impossible to tell at what stage this parasite attacks the Syrphid.

Mr G. E. J. Nixon of the British Museum (Natural History) kindly identified the genus.

Museum for identifying and confirming the genus *Syrphophagus*. I am also indebted to the authorities of the British Museum, particularly Dr J. Smart, for allowing me to examine some Syrphid puparia in the Museum collection.

REFERENCES

FAURE, J. C. (1923). Mode de succion de *Pachyneuron* sp. Chalcidien parasite des pupes de *Syrphus balteatus*. *Rev. Path. vég.* **10**, 255.

KELLY, E. O. G. (1914). Notes on the biology of *Diplazon* (*Bassus*) *laetatorius*. *J. econ. Ent.* **7**, 294.

MAYR, G. (1876). Die europäischen Encyrtiden. *Verh. zool.-bot. Ges. Wien.* **25**, 675.

METCALF, C. L. (1911). Life histories of Syrphidae. 2. *Ohio Nat.* **11**, 397.

—— (1912). Life histories of Syrphidae. 3. *Ohio Nat.* **12**, 477, 533.

—— (1913). Life histories of Syrphidae. 5. *Ohio Nat.* **13**, 81.

—— (1916). Syrphidae of Maine. *Bull. Me agric. Exp. Sta.* no. 253, p. 193.

—— (1917). Syrphidae of Maine (2nd report). *Bull. Me agric. Exp. Sta.* no. 263, p. 153.

MORLEY, C. (1911). *Ichneumonologia britannica*, **4**. London.

RICHARDS, O. W. (1930). A proctotrupid bred from a Syrphid pupa. *Ent. mon. Mag.* **76**, 277.

SALT, G. (1936). Miscellaneous records of parasitism. *Ent. mon. Mag.* **72**, 9.

THORPE, W. H. (1930). Observations on the parasites of the pine shoot moth, *Rhyacionia buoliana* Schiff. *Bull. ent. Res.* **21**, 387.

VOUKASSOVITCH, P. (1925). Observations biologiques sur quelques insectes prédateurs des Pucerons et leurs parasites et hyperparasites. *Bull. Soc. ent. Fr.* p. 170.

EXPLANATION OF PLATES XXV–XXX

PLATE XXV

Figs. 1–8. *Syrphus luniger* Mg.   Fig. 1. Side view of puparium.   Fig. 2. Dorsal view of puparium.   Fig. 3. Antero-dorsal view of posterior spiracular trunk.   Fig. 4. Postero-ventral view of posterior spiracular trunk.   Fig. 5. Lateral view of posterior spiracular trunk.   Fig. 6. Upper surface of chorion, H.P.   Fig. 7. Upper surface of chorion, L.P.   Fig. 8. Lower surface of chorion, H.P.

Figs. 9, 10. *Syrphus ribesii* (L.).   Fig. 9. Dorsal view of chorion, H.P.   Fig. 10. Lateral view of dorsal edge of chorion, H.P.

PLATE XXVI

Fig. 1. *Syrphus ribesii* (L.). Side view of puparium.

Fig. 2. *Syrphus torvus* O.-S. Side view of puparium.

Figs. 3–7. *Syrphus auricollis* Mg.   Fig. 3. Side view of puparium.   Fig. 4. Dorsal view of puparium.   Fig. 5. Posterior view of posterior spiracular trunk.   Fig. 6. Lateral view of posterior spiracular trunk.   Fig. 7. Dorsal view of chorion, H.P.

Figs. 8–10. *Syrphus cinctellus* (Zett.).   Fig. 8. Lateral view of puparium.   Fig. 9. Posterior view of posterior spiracular trunk.   Fig. 10. Lateral view of posterior spiracular trunk.

PLATE XXVII

Fig. 1. *Syrphus cinctellus* (Zett.). Antero-dorsal view of posterior spiracular trunk.
Figs. 2–6. *Syrphus albostriatus* (Fln.). Fig. 2. Dorsal view of puparium. Fig. 3. Lateral view of puparium. Fig. 4. Antero-dorsal view of posterior spiracular trunk. Fig. 5. Dorsal view of posterior spiracular trunk. Fig. 6. Lateral view of posterior spiracular trunk.
Figs. 7–10. *Syrphus balteatus* (Deg.). Fig. 7. Dorsal view of puparium. Fig. 8. Lateral view of puparium. Fig. 9. Lateral view of posterior spiracular trunk. Fig. 10. Postero-dorsal view of posterior spiracular trunk.

PLATE XXVIII

Figs. 1–4. *Catabomba selenitica* (Mg.). Fig. 1. Lateral view of puparium. Fig. 2. Posterior view of posterior spiracular trunk. Fig. 3. Postero-ventral view of posterior spiracular trunk. Fig. 4. Lateral view of posterior spiracular trunk.
Figs. 5–8. *Catabomba pyrastri* (L.). Fig. 5. Lateral view of puparium. Fig. 6. Antero-dorsal view of posterior spiracular trunk. Fig. 7. Lateral view of posterior spiracular trunk. Fig. 8. Posterior view of posterior spiracular trunk.
Figs. 9–11. *Baccha elongata* (Fab.). Fig. 9. Lateral view of puparium. Fig. 10. Dorsal view of posterior spiracular trunk. Fig. 11. Dorsal view of puparium.

PLATE XXIX

Figs. 1, 2. *Platychirus scutatus* (Mg.). Fig. 1. Side view of puparium. Fig. 2. Dorsal view of posterior spiracular trunk.
Fig. 3. *Platychirus albimanus* (Fab.). Upper surface of chorion, H.P.
Figs. 4–7. *Sphaerophoria scripta* (L.). Fig. 4. Lateral view of puparium. Fig. 5. Dorsal view of puparium. Fig. 6. Lateral view of posterior spiracular trunk. Fig. 7. Postero-dorsal view of posterior spiracular trunk.
Fig. 8. *Sphaerophoria flavicauda* Zett. Lateral view of puparium.

PLATE XXX

Fig. 1. *Bassus laetatorius* (Fab.). Mouth-parts of mature larva.
Fig. 2. *Homocidus signatus* (Grav.). Mouth-parts of mature larva.
Fig. 3. *Homocidus tarsatorius* (Pz.). Mouth-parts of mature larva.
Fig. 4. *Promethus monticola* Sey. Mouth-parts of mature larva.
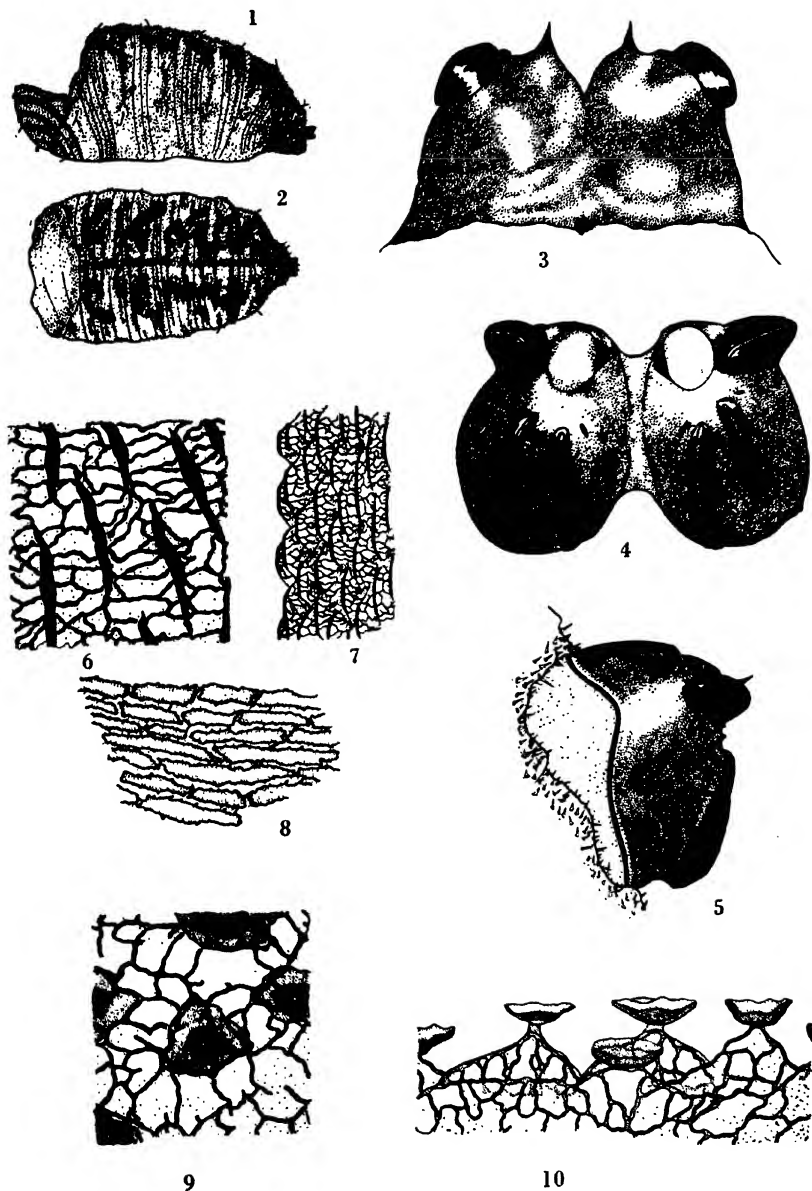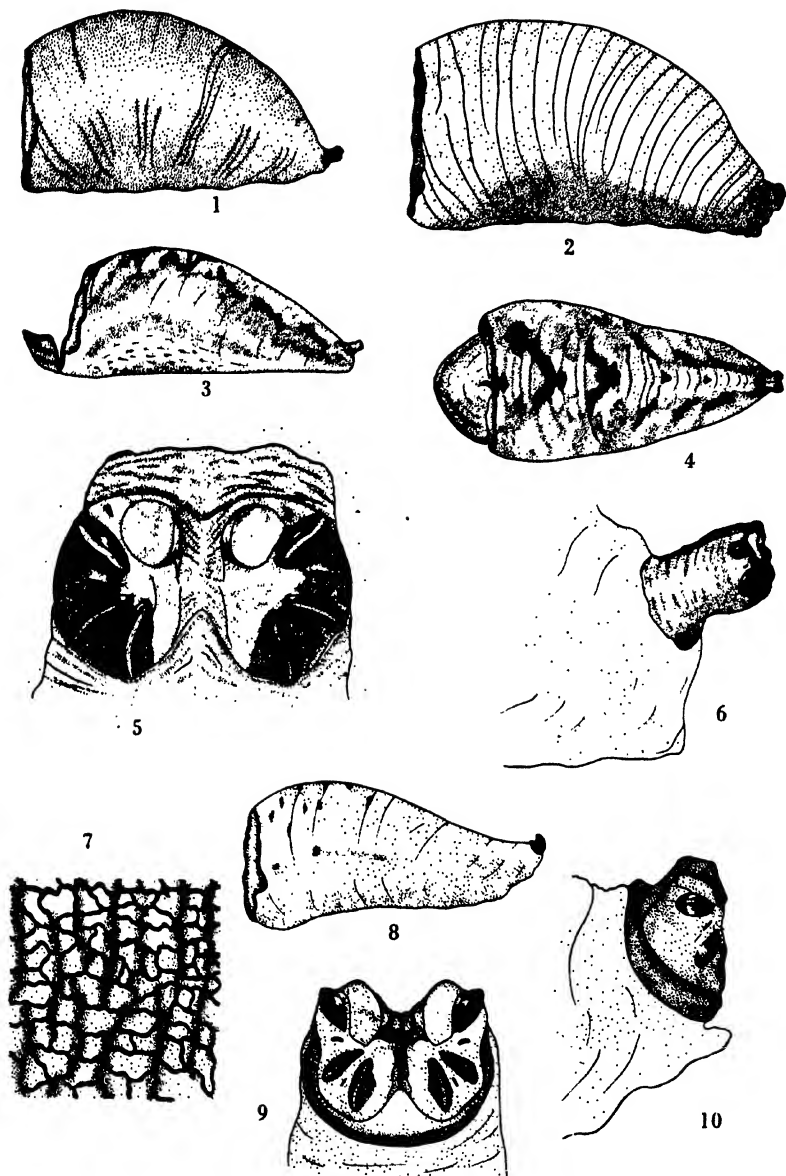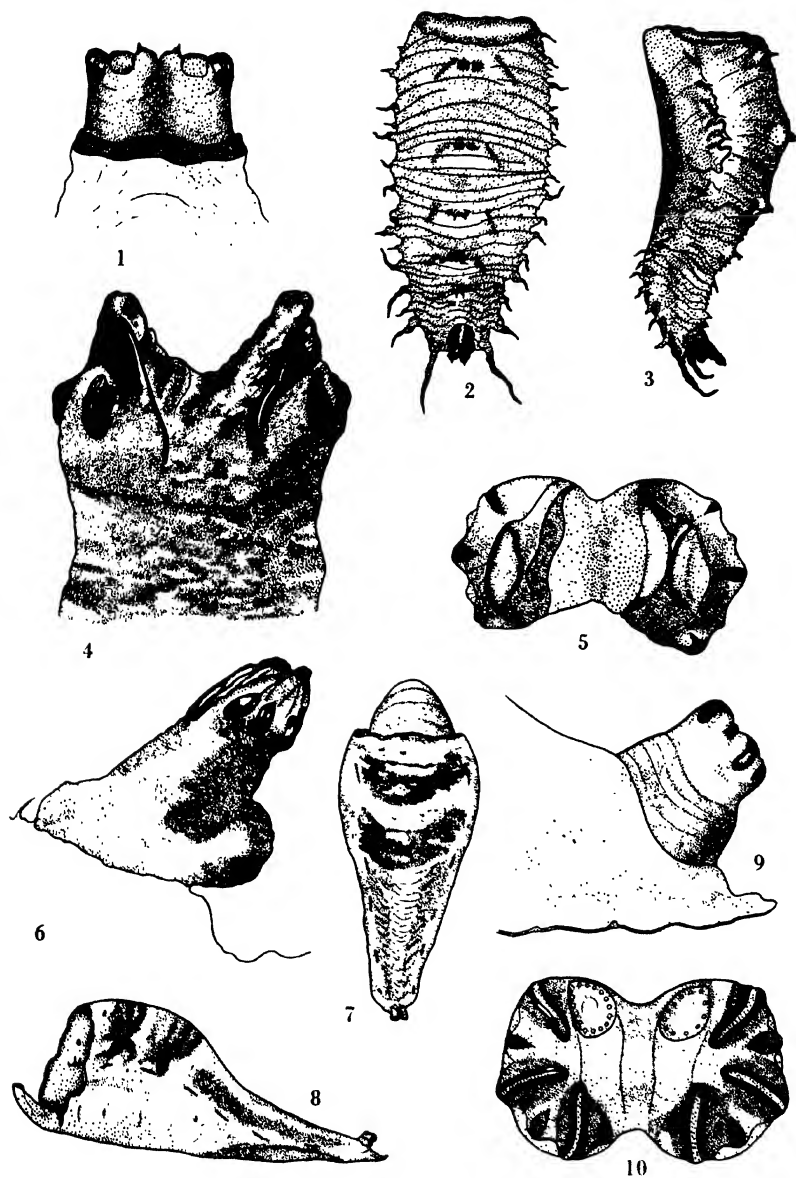
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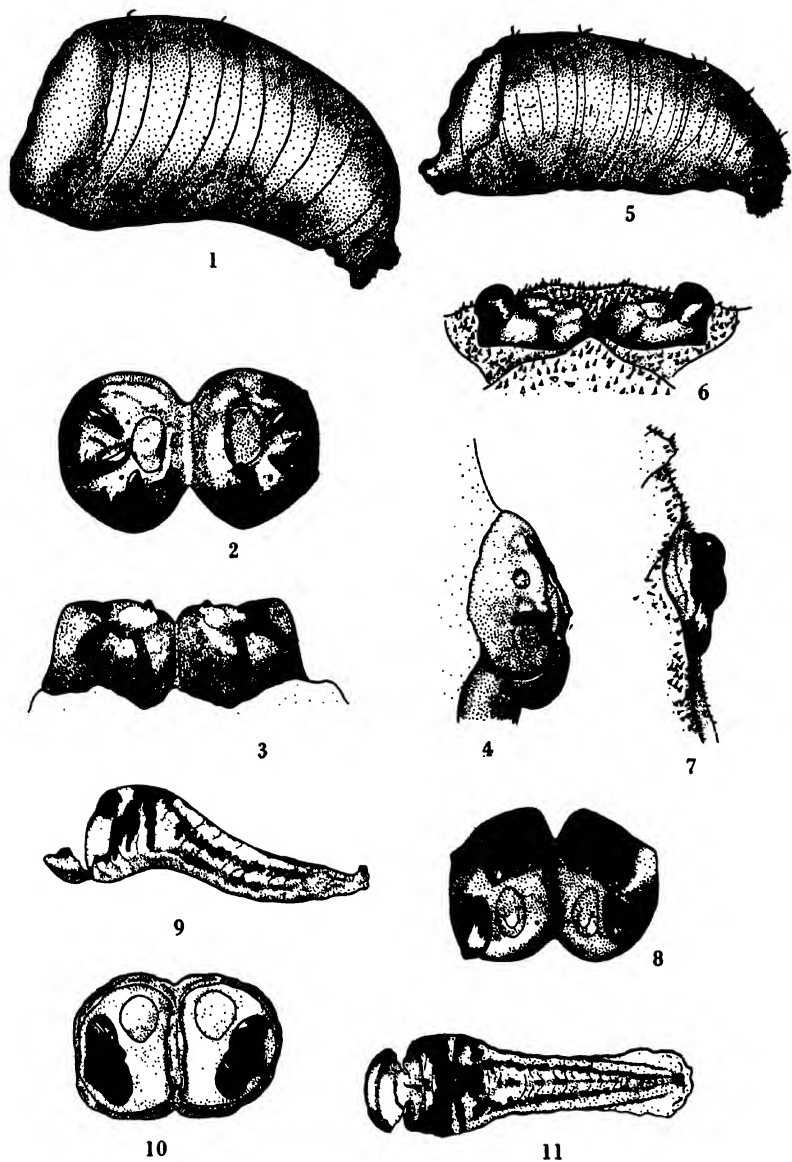
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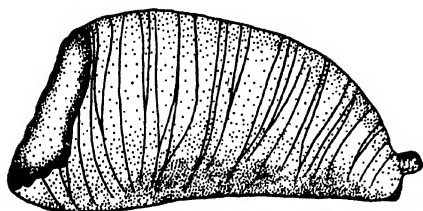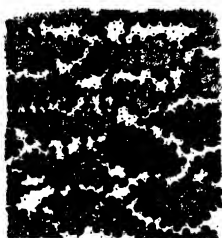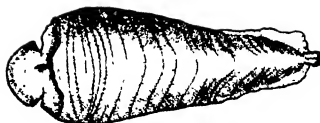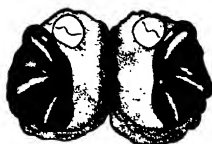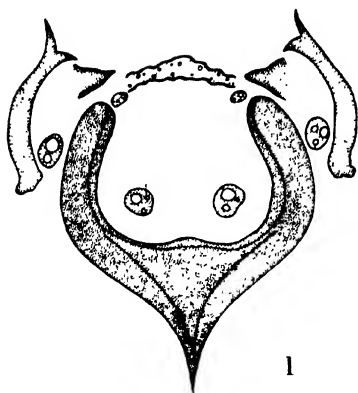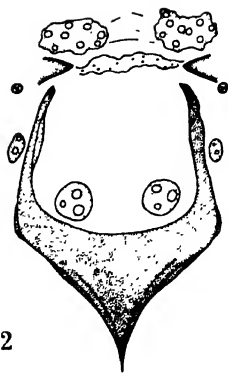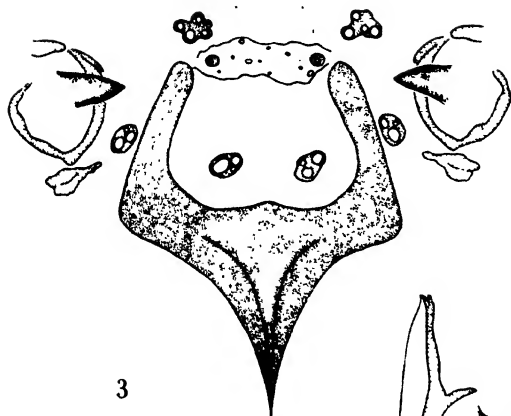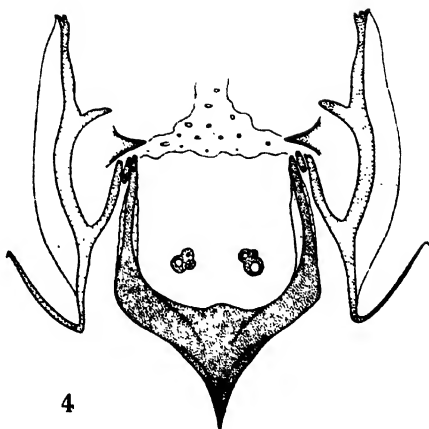
FACTORS AFFECTING THE RESISTANCE OF THE FLOUR BEETLE, *TRIBOLIUM CONFUSUM* DUV., TO HYDROGEN CYANIDE[1]

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(With Plates XXXI and XXXII and 10 Text-figures)

CONTENTS

1. INTRODUCTION

MUCH work has been carried out in recent years on the effect of various environmental factors on the resistance of insects to fumigants. Some of the main lines of this work have been summarized in papers by Potter (1937) and Shepard *et al.* (1937). The variation in resistance of an insect

[1] Thesis approved for the degree of Doctor of Philosophy in the University of London.

with variation in its physiological state has also been studied. Knight (1925) stated that insects become more resistant to hydrogen cyanide when they become dormant or inactive, however this condition is brought about. A number of workers have shown that the resistance of an insect is different at different stages in its life history and their results are discussed in § 4 of this paper. It was proposed to investigate in detail this difference in resistance at different stages, particularly considering the application to it of Cotton's theory of the relation of resistance to fumigants and respiratory metabolism (Cotton, 1932). During the course of this work interesting problems arose which were followed up as far as time permitted.

The flour beetle, *Tribolium confusum* Duv., was chosen as a test insect. It has a short life cycle, can easily be reared under controlled conditions of temperature and humidity, and the various stages can be sieved out of the flour on which it feeds. Hydrogen cyanide was found to be a convenient fumigant, as it could be obtained very nearly pure, and a method had been established for its determination in low concentrations (Lubatti, 1935). Also, its physiological action on cellular respiration by inhibiting oxidation of the respiratory enzymes is well known from the work of Warburg, Keilin and others (Meldrum, 1933).

2. DESCRIPTION OF APPARATUS USED

(i) *The first apparatus*

The apparatus with which most of the work was carried out was designed by Dr H. H. S. Bovingdon and is a modification of that described by him (Bovingdon, 1934). It is housed in a constant-temperature cabinet (Pls. XXXI and XXXII). This cabinet is made up of a framework of $2 \times 1\frac{1}{2}$ in. battens covered internally and externally with match-boarding, the intervening space being filled with slag-wool. The upper part of the cabinet is removable and has two double-glass windows in the top, and two felt-covered apertures on each side. The stopcocks can be manipulated through these apertures. The door in the lower part of the cabinet permits access to the tubes to which the sampling flask and the evaporating chamber are attached. The bubblers for controlling the humidity of the air in the apparatus are on the floor of the cabinet.

A diagram of the apparatus is shown in Text-fig. 1. This system and an identical one as a control are supported on a wooden framework. This is suspended about a metal rod (pivot in diagram) which is fixed to the cabinet so that by means of a crank worked by an electric motor the whole apparatus can be rocked slowly from side to side. The motor is geared down so that the framework is rocked backwards and forwards through an arc of about 15° about twice a minute. The mercury reservoir and the manometer are outside the cabinet and do not move with the rest of the apparatus.

X and Y are two large glass flasks of approximately 3 and $1\frac{1}{2}$ l. capacity respectively. These form the main gas reservoir and flask X, in addition, acts as a mixing

chamber. They are connected by a tube at their lower ends and contain about 150 c.c. mercury. At the top they are connected by a straight tube and also through the insect chamber. The latter is a hollow glass sphere, about 100 c.c. capacity, closed by a ground-glass stopper (not shown in the diagram), and with two side tubes each supplied with a two-way stopcock. As the apparatus is rocked from side to side the mercury rises and falls alternately in the two flasks X and Y and pumps the gas backwards and forwards through the insect chamber or the bypass, so that there is a constant circulation of gas. To avoid one pocket of gas going backwards and forwards, the tube from the insect chamber is continued half-way down the large flasks.
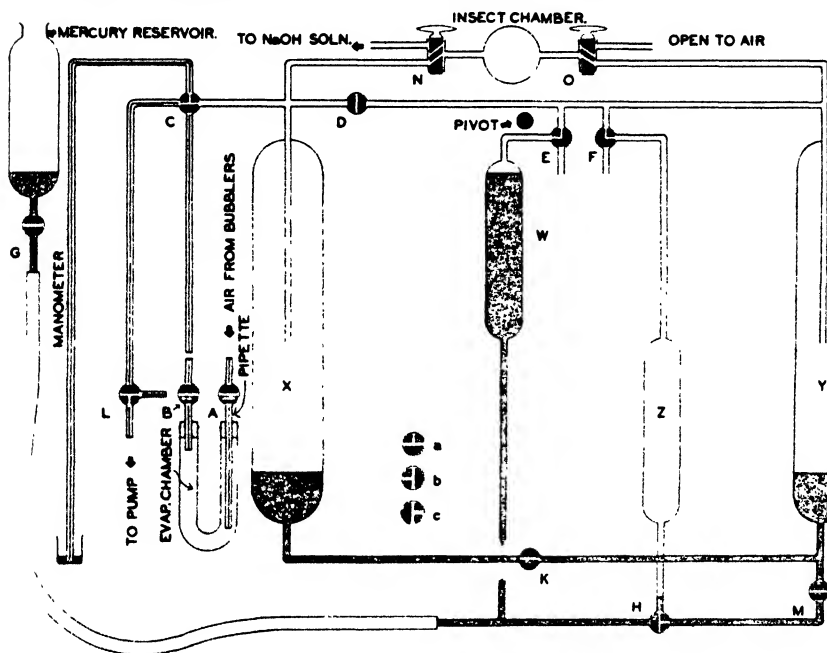
Text-fig. 1. Diagram of first apparatus. (See text for explanation.)

By allowing mercury to flow from the outside reservoir (which can be raised and lowered) into flask W, and from W to Z, the pressure in the apparatus can be restored to atmospheric after removing a sample of the gas for analysis, and by turning the appropriate stopcocks the insect chamber can be flushed out with the fumigant.

All the flasks and connecting tubes and stopcocks are of glass, and, where joins are necessary, butt connexions are made and joined with rubber pressure tubing. With the exception of about 3 in. of rubber tubing between the apparatus and the manometer no rubber is actually exposed to the gas. The apparatus is vacuum-tight and a Gaede rotary oil pump is used to evacuate it.

The hydrogen cyanide is measured in small graduated pipettes exactly as described by Bovingdon. The pipette is so calibrated that quantities of liquid hydrogen

cyanide corresponding to a range of concentrations of from 0·06 to 2·0 mg./l. can be measured out roughly. This process is referred to throughout this paper as "dosing". The accurate measurement of the gas concentration is described later.

The apparatus is heated by electric resistance heaters controlled by a bimetallic thermostat.

The temperature of the insect chamber did not vary more than $\pm 0.2°$ C. during an experiment. The humidity is controlled by passing the air entering the apparatus through six gas-washing bottles containing either potassium hydroxide or sulphuric acid solution. These solutions are made up to a certain specific gravity which will give the air the desired humidity. The specific gravity of these solutions is checked at intervals and if necessary they are replaced. When sulphuric acid was used as the principal means of controlling the humidity, two extra bottles of potassium hydroxide were added to absorb carbon dioxide so that these experiments might be comparable with those in which potassium hydroxide alone was used. The first four gas-washing bottles were ordinary Dreschel bottles. The next two had sintered glass bubblers, and these were followed by an empty Dreschel bottle and a tube of glass-wool to trap any spray.

The circulation of the gas was tested by attaching a bubbler—previously calibrated—to the open end of the insect chamber and observing the number of bubbles passing per minute in one direction. The rate of flow was about 7 l./hr., which corresponded to a linear velocity through the insect chamber of the order of 5 m./hr.

A sample of the gas in the apparatus was taken at the beginning and end of each test. The sampling flask (shown attached to the apparatus in Text-fig. 2) was a cylindrical glass vessel about 3 cm. in diameter and with a capacity of about 120 c.c. A right-angle stopcock, with a removable key, is fused to one end of the flask so that it could be opened or closed to the apparatus or vacuum pump, and afterwards the key could be removed and titration carried out directly into the flask.

Procedure of each test.

(1) All mercury is run out of flasks W and Z, by opening stopcock G, lowering the mercury reservoir and turning stopcocks E and F to open flasks W and Z to the air.

(2) Stopcocks G, N, K and M are closed; O is open to the air, D is opened, C is turned to position (a), L to position (b), H to position (c), and E and F turned so that flasks W and Z are in communication with the rest of the apparatus, which is now evacuated. Meanwhile, the hydrogen cyanide is taken up in the small pipette and the latter inserted in the evaporating chamber and cocks A and B are closed. The chamber is then placed in position, one tube being connected to the apparatus and the other to the bubblers; both connexions are made with butt joints connected with rubber pressure tubing.

(3) Stopcock C is turned to position (b) so that the tube B–C is evacuated; D is closed, L is turned to the position shown in the diagram, and C is turned to position (c). B is opened and the hydrogen cyanide, volatilized under the reduced pressure, is admitted to the flask X. The gas in this flask is then brought to atmospheric pressure by slowly opening stopcock A and admitting air of the desired humidity.

(4) Stopcock A is closed and D is opened, thus admitting the well-mixed gas in flask X to the rest of the apparatus. Air is again allowed to enter by opening stopcock

A until the whole apparatus contains a gas mixture at atmospheric pressure. Stopcock B is then closed.

(5) The insects, previously placed in their container, are suspended from a hook in the stopper of the insect chamber. Stopcock K is opened and the rocking motor started. An interval of about 15 min. is allowed for the mixing of the gas and for the insects to settle down whilst still in air.

(6) The rocking motor is stopped: stopcock D is closed and O and N turned so that there is now a passage through the insect chamber from flask Y to a bottle containing a strong solution of sodium hydroxide. Stopcock G is opened and the reservoir raised so that mercury flows from the latter to flask W. The air in the insect chamber is thus displaced by the fumigant. As flask W has approximately four times the volume of the insect chamber it may be assumed that the gas mixture in the latter is similar in composition to that in the rest of the apparatus. This operation takes about a minute. Stopcock N is then turned so as to put the insect chamber and flask X in communication, the rocking motor is started and the time is noted. An ordinary watch is used to time the tests. The stopcocks are now in the positions shown in Text-fig. 1.

(7) The gas-sampling flask, containing 5 c.c. of $N/20$ sodium hydroxide, is attached to the side tube of cock L, stopcock C is turned to position (b), L to position (c) and the evacuating pump switched on. When the manometer indicates a pressure of only a few millimetres of mercury, stopcock L is turned to position (a) and C is also turned very slowly to position (a), so that gas from the apparatus enters the sample flask. The latter is then closed by turning the key and the pressure of the gas noted on the manometer. Mercury is then run from flask W into flask Z by turning stopcock E so as to open W to the air and turning H to position (b). When atmospheric pressure is restored, H is turned back to position (c), C is turned to position (c) and the sampling flask removed. After the addition of 2 c.c. of a 7 % solution of sodium bicarbonate the sodium cyanide is titrated with standard iodine solution using 0·5 c.c. benzene as indicator.

(8) At the end of the test the rocking motor is stopped, stopcocks O and N are closed and the insects removed. A second gas sample is taken at the end of each test. A similar procedure to that described in paragraphs (1) and (2) is followed and the apparatus is evacuated and air readmitted.

Except that the hydrogen cyanide was omitted, the control insects were given exactly the same treatment.

The concentration of hydrogen cyanide in mg./l. is calculated from the mean of the two titrations. Usually there was a loss of about 1–5 % of hydrogen cyanide due to absorption by the insects, their container and stopper-grease. If the loss exceeded this, the test was not usually included in the working out of results. Certain tests, however, unavoidably gave a very high loss. These are referred to later (§ 7).

Accuracy of titrations of the sodium cyanide.

Two solutions of iodine were used, one containing about 0·5 and the other about 0·25 mg./l. The accuracy of this titration ranged from about ±6 % at the lowest concentrations to less than ±1 % at the highest.

<center>(ii) *The second apparatus*</center>

The apparatus described above had certain disadvantages, and for later work it was replaced by one in which some of these disadvantages were overcome. The chief of these were:

(1) Its small volume (5·5 l.) made accurate dosing difficult, and even normal losses of hydrogen cyanide formed quite a large proportion of the total amount of hydrogen cyanide present.

(2) The number of stopcocks and interconnexions made it difficult to clean and necessitated several butt joints covered with rubber tubing. The large amount of mercury was expensive, and also laborious to clean. Also it was later found to be toxic to insects (Gough, 1938b).
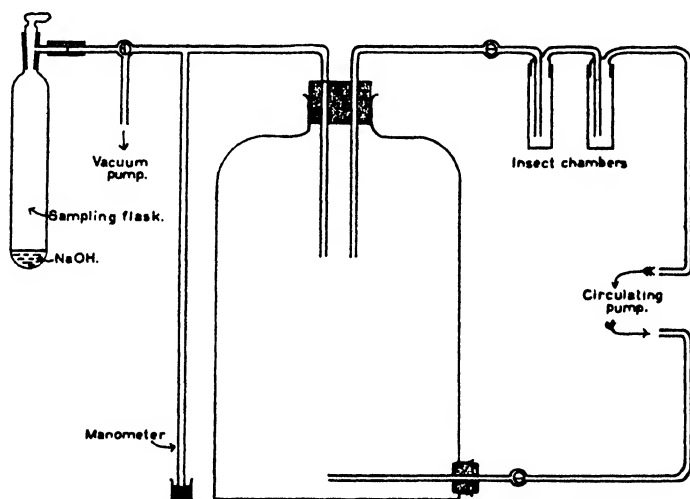
Text-fig. 2. Diagram of second apparatus. (See text for explanation.)

A diagram of the second apparatus is shown in Text-fig. 2. It was used for all the experiments on the sixth and seventh filial generations, described in § 5, and all the experiments described in § 6. It was built up in a constant temperature and humidity room, so that there was no need to control these factors further. In order to remove carbon dioxide from air entering the apparatus, the air was bubbled through a solution of potassium hydroxide of such a strength that the humidity was not altered. A 20 l. aspirator formed the main gas reservoir. This was sufficiently large to minimize the proportional losses of hydrogen cyanide caused by the presence of adult beetles (see § 7). Owing to the possibility of breakage it was not advisable to evacuate it completely, so that after each test it was half evacuated and refilled with air four times.

The top and bottom openings of the aspirator were closed with a rubber bung covered with tinfoil so as to prevent the absorption of hydrogen cyanide. Each carried a glass tube leading to the insect chambers and the mercury circulating pump. The latter, a modified Leach type of pump, contained only a small quantity of mercury,

and this could be covered with some inert substance if necessary. This was not done as it was not realized at this stage that the mercury vapour might be toxic. The insect chambers were small gas washing bottles of about 15 c.c. capacity. They could be shut off from the rest of the apparatus by stopcocks. A second tube in the top bung led to the manometer and to a threeway cock. The sampling flask was attached to one arm of this and the other arm was either connected to the evacuating pump or could be used for the attachment of the evaporating chamber.

Procedure of experiment.

The aspirator was first partially evacuated, the cocks to the circulating pump being closed. The hydrogen cyanide was then admitted, using the same method as described above, and air swept through the evaporating chamber to bring the apparatus to atmospheric pressure. The circulating mechanism was then started after the appropriate stopcocks had been opened. After about half an hour the pump was stopped, the stopcocks closed and the insects placed in the insect chamber, which already contained hydrogen cyanide. Some of this was inevitably lost whilst the insects were being introduced. The small size of the chambers and the relative efficiency of the pump, ensured that the gas concentration in the insect chambers would reach that in the rest of the apparatus within a few minutes of the circulating pump being restarted. This arrangement, of course, did not give the insects the opportunity of settling down. Gas samples were taken exactly as described for the first apparatus. Control experiments had to be carried out in the same apparatus when fumigations were not being performed: they were only done occasionally.

3. TECHNIQUE

(i) *The breeding of large numbers of insects of known stage, age and sex*

The beetles were bred on a roller-ground wholemeal flour, which was sieved through no. 9 bolting silk. The flour was first sterilized and then exposed on shallow trays in the constant temperature and humidity room for about a fortnight. During this period, which allowed it to come into equilibrium with the conditions in the room, it was covered with muslin to prevent reinfestation. All the cultures were kept in this room at 27° C. and 60 % relative humidity, and all fumigation tests were carried out under these conditions.

The following technique was employed to obtain the various stages at a known age. The figures in brackets refer to the approximate duration of the stage in days.

Eggs (6). Cultures of about 200 adults, between 1 and 2 months old, were made up in glass jars, about half full of flour. The adults were removed every day by means of a coarse sieve and the flour then sieved through no. 6 bolting silk, which retained the eggs. The adults were then replaced in the flour, which was changed once a week. These cultures were never kept more than 1 month. From one of these cultures it was possible to obtain about 300 eggs per day.

Larvae (24). Eggs laid within 24 hr. of one another, were made up into cultures, so that the age of the resulting larvae was known within a day or so, since nearly all the eggs hatched on the same day. The larvae were fumigated 26 days after the eggs were laid, or 20 days after hatching. At this age the larvae are in the last instar, but they are still active and cling to paper when placed on it. A day or so later, in the pre-pupal period, they become inactive and do not cling to paper. It is not known for

certain whether they had ceased feeding at that age, although nearly all the controls pupated successfully. Owing to individual variation in rate of development, the larvae from one culture were not all in the same instar on the day they were to be fumigated, but it was not difficult to select only active larvae, in the last instar. It was more satisfactory to do this, using a separate culture for each test, rather than selecting from a mass culture, since no individuals, which might be used for future tests, were disturbed.

Pupae (8). About 200 adults were placed in a large glass jar, about one-third full of flour, and allowed to lay eggs. After a fortnight, the adults were removed, and the eggs and those larvae which had already hatched left to develop. When the pupae formed they were sieved out daily with a coarse sieve, and sorted from the larvae, which were replaced. The pupae were then divided into males and females, and, either fumigated immediately, or left in an open tube in the constant temperature room until they were required.

Adults (several months). When 0–1-day-old adults were required, several hundred pupae of all ages were divided into males and females and the resulting adults fumigated together, but in separate containers, on the day of emergence. Several attempts were made to mark the adults after emergence from pupae of known sex so that they could be easily separated again after mating. The only satisfactory method, of those tried, was the removal of the hind tarsi from one leg in one sex, and from the opposite leg in the other. This operation, which was carried out several days before fumigation, took a long time if several hundred beetles were required, and it was open to the usual objections to the mutilation of experimental animals. Finally, it was found very easy to sort the sexes after fumigation, by gently squeezing the end of the abdomen with a pair of forceps and examining the extruded genitalia under a binocular microscope or even with the naked eye. In dead or stupefied females the genitalia were frequently extruded after fumigation. Usually, this examination was carried out the day after fumigation, but if it had to be delayed for a few days so that the dead beetles dried up, the same process could be performed after boiling the insects in a weak solution of potassium hydroxide.

(ii) *The gross effect of hydrogen cyanide and the criterion of death in each stage*

Hamlin & Reed (1927) drew attention to the necessity of keeping fumigated insects under observation for some time after fumigation, the period depending on the insect. Many fumigated insects, which are apparently dead, recover later and others, which are apparently unaffected, may die later. This second possibility was not observed to occur in the present work.

After fumigation, the test insects and controls were transferred to tubes in the constant temperature room. Larvae and adults were supplied with flour, and a small piece of paper was put in with the eggs and pupae so that the resulting larvae or adults could crawl upon it.

Eggs. A certain percentage of the control eggs failed to hatch. This proportion varied from 0 to 36 %, with an average of 17 % in the 0–1-day-old eggs, and from 3 to 25 %, with an average of 10 % in the 3–4-day-old eggs. Because, sometimes, at low concentrations more eggs survived among those fumigated than among the controls, instead of correcting for the percentage survival of controls in each batch of eggs, it was decided to use the average percentage survival when computing the net kill in

each test. The following method, suggested by Bliss (1935 a), was used for this purpose. The number of individuals used in a particular test is multiplied by the proportion alive in the untreated controls. This gives the net number of insects exposed to the poison. When the number surviving the treatment is subtracted from this net total, the difference is the number of killed, and the number killed, multiplied by 100, and divided by the net number exposed is the percentage killed. About 100 eggs each were counted out for the fumigation and control tests. When the young larvae hatched they were removed and counted daily. Occasionally a larva hatched but died afterwards: this was not included among the survivors. There was a delay of 1-3 days in the hatching of these survivors compared with the controls.

Larvae. About sixty larvae were used in each test, another sixty from the same culture being used as controls: there was no mortality among the latter. As the test larvae were ready to pupate, those which formed an apparently normal pupa were counted as survivors. The larvae which died were torpid for a day or so after fumigation. Subsequently dark blotches appeared in various places, usually starting on the thorax and spreading over the whole body, until a black shrivelled skin was left. No larvae, which were active immediately after fumigation, died later.

Pupae. About sixty pupae were used in each test and an equal number of controls. Frequently pupae which had been fumigated, gave rise to adults in which the elytra were undeveloped and which were only able to move their legs feebly and, because of this, only pupae which formed an apparently normal adult, capable of walking normally, were counted as survivors. No account was taken of a slight mortality of about 1-2 % in the controls. The emergence of adults from pupae which survived fumigation was delayed from 1 to 7 days. Some rough experiments were carried out on the egg production of these survivors, and it appeared that they at first produced only a few eggs and those not viable. After 2 or 3 weeks they produced viable eggs in normal numbers.

Adults. There was no mortality amongst the control adults, except for one lot which are dealt with in § 7 of this paper.

At the time of fumigation, none of the 0-1-day-old adults had darkened to the rich brown colour typical of older beetles. Beetles which had only just emerged and in which the elytra were still white were not used. After even the lowest concentrations to which the 0-1-day-old adults were exposed, all were stupefied at the end of the fumigation. The survivors gradually recovered about 3-7 days afterwards.

The older adults, 14 and 28 days old, behaved quite differently from the young ones. At the end of a test it was almost possible to sort out those which were alive and those which were dead, and only one or two of those supposed dead recovered later. Immediately after the fumigation, the survivors frequently were unable to co-ordinate their walking movements though they became normal on the following day. Whether this difference between young and older adults is due to a difference of age or to a difference in the method of experiment is discussed in the next section.

(iii) *Containers used for the insects during fumigation*

Several different types of containers were used to hold the insects during the fumigation: these included bags of muslin or bolting silk and short lengths of glass tubing with each end covered with bolting silk. Eventually, cylindrical iron-wire gauze cages, about 1 in. long and 0.5 in. in diameter, were found to be most con-

venient. They were strong, non-absorptive and had a large surface to which the insects could cling. Their mesh, 20 to the inch, was sufficiently wide not to affect diffusion and yet sufficiently close to ensure the retention of all stages except the eggs. For these, very small bags, made of no. 6 bolting silk, were used.

(iv) *Analysis of results*

Bliss's method (1935 *a*, *b*) was used in the present paper to compare the resistance of different stages or groups of insects. All the tests were performed at 27° C. and 60 % relative humidity, and 1¼ hr. was the standard time of exposure.

As never more than about 100 eggs were used in each test, and rarely more than sixty individuals of any other stage, it was thought unnecessary to work out the percentage mortality to more than the nearest 1 %. When converted into probits the first three significant figures were taken. Although at very high percentage kills the third figure of the probit value varied quite considerably with a 1 % difference, it was thought best to be consistent, especially as tests at high concentrations had very little weight.

Throughout this paper the word "test" has been used to describe the exposure of a certain number of insects of a given stage under given conditions to a certain concentration of fumigant. A number of such tests on the same stage, under the same conditions, has been called an experiment. It should also be noted that the word "sample" refers to a sample of the gas mixture to which the insects were exposed and not to a sample of the insect population.

4. RELATIVE RESISTANCE OF DIFFERENT STAGES TO HYDROGEN CYANIDE

Table I and Text-fig. 3 summarize the results of experiments performed to determine the relative resistance of different stages of the flour beetle to hydrogen cyanide. Graphs showing the relationship of (1) percentage mortality and concentration of hydrogen cyanide and (2) mortality expressed as probits and log concentration have been plotted in Text-figs. 6–8, and regression lines for the probit/log concentrations have been calculated. The concentrations corresponding to a 50 % kill for each stage investigated have been computed, together with their limits of error, from equation (28) of Bliss's paper (1935 *b*). The statistic "*t*" in this equation was read from Fisher's Table III (Fisher, 1932) at $P = 0.05$. The limits of error are indicated in Text-fig. 3 by the differences in length of the boundaries of each black area.

If the value for χ^2 is smaller than the value corresponding to a probability of 0·05 in Fisher's Table III the data may be considered consistent with the straight line that has been fitted to them. If the value for χ^2 is greater than the value corresponding to a probability of 0·05 the variation about the line that has been fitted is significantly greater than would be expected from simple fluctuation in sampling, and this is denoted in the table by S.
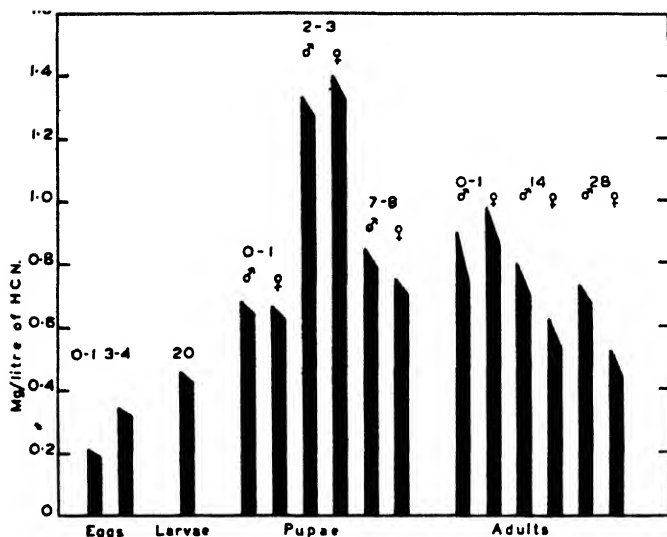
Text-fig. 3. Concentrations of hydrogen cyanide necessary to give a 50 °₀ kill of different stages of *Tribolium* at 27° C and 60 °₀ relative humidity for 1½ hours. The limits of error within which the concentration for each stage has been determined are indicated by the differences in length of the boundaries of each black area. The figures above each stage refer to the age of that stage in days.

Table I. *Summary of data of experiments on different stages*

Stage		n	χ^2	Mg. l. of hydrogen cyanide to give 50 % kill
0–1-day-old eggs		12	48·7128 (S.)	0·195 ± 0·007
3–4-day-old eggs		13	70·7716 (S.)	0·326 ± 0·007
20-day-old larvae		15	126·4629 (S.)	0·439 ± 0·015
0–1-day-old pupae:	Male	12	103·2521 (S.)	0·659 ± 0·015
	Female	8	68·9811 (S.)	0·639 ± 0·018
2–3-day-old pupae:	Male	13	126·8112 (S.)	1·296 ± 0·023
	Female	11	64·6612 (S.)	1·363 ± 0·035
7–8-day-old pupae:	Male	17	217·5798 (S.)	0·817 ± 0·022
	Female	19	324·7490 (S.)	0·729 ± 0·020
0–1-day-old adults:	Male	6	4·6915	0·810 ± 0·085
	Female	8	8·3724	0·919 ± 0·060
14-day-old adults:	Male	5	4·8909	0·750 ± 0·031
	Female	5	32·9446 (S.)	0·569 ± 0·038
28-day-old adults:	Male	9	29·2491 (S.)	0·703 ± 0·025
	Female	8	30·7116 (S.)	0·479 ± 0·040

The limits of error above and below the mean are not necessarily quite the same. For convenience the results are expressed as ± the greater error.

Cotton (1932) states that in most holometabolic insects, the adult has the highest rate of metabolism, followed, in order, by the larva and the pupa. In support of this the only data quoted were those of Batelli and Stern on flies. Cotton demonstrated experimentally that the order of resistance to carbon disulphide of different stages of *Tribolium confusum* Duv. and *Ephestia kühniella* Zell. was pupa (most resistant), larva, adult. He deduces from this, that, other conditions being equal, a difference in respiratory rate between the various stages corresponds to a difference in susceptibility to fumigants. This suggestion has been investigated in more detail by Lindgren (1935), who studied the order of resistance of different stages of *Tribolium* to various fumigants, with the following results:

Carbon disulphide: Egg—pupa—adult—larva (low humidity).

Pupa—egg—adult—larva (high humidity).

Chloropicrin: Egg—pupa—adult—larva.

There was little difference between the resistance of the adults and larvae towards either of these fumigants.

Ethylene oxide: Pupa—adult—larva—egg.

All the above refer to tests on groups of individuals of various ages. The present writer found the following order of resistance to *hydrogen cyanide*: pupa—adult—larva—egg. This is true if the most resistant age tested of both pupae and adults be taken as representative of those stages.

If the egg be neglected, the order of resistance to all these poisons is pupa—adult—larva. According to Lindgren (1935) the following is the order of the rate of metabolism of the different stages (Highest first):

Larva—adult—pupa—egg (without flour).

Old adult—larva—young adult—pupa—egg (with flour).

In the table in which he compared respiratory rates to susceptibility to fumigants, Lindgren showed the figures of respiratory rates referring to insects with flour, though, as his fumigations were presumably carried out on insects without flour, it would seem more reasonable to use the corresponding respiratory rates. If this be done and the egg is not included, there is a correlation between respiratory rate and susceptibility to fumigants, that is, the stage with the highest metabolism is least resistant. Without further work in which fumigation tests and measurement of respiratory rates are performed under exactly the same conditions, and on insects from the same stock, it would be unwise to be

dogmatic on this point. It must be remembered, however, that (1) rate of metabolism is usually measured per gram live weight, whereas resistance is usually measured independently of size, (2) that the rate of metabolism is increased enormously by increase in activity. It may, however, probably be considered significant that in *Tribolium*, the order of resistance, pupa—adult—larva, is common to a number of fumigants. It is interesting to note that in *Tribolium*, the egg, which has the lowest rate of metabolism, is sometimes the most, and sometimes the least resistant stage. It may be possible to group fumigants according to this differential action on the egg and on the other stages. Gough (1938a) has pointed out that the egg of the bed-bug is more resistant to sulphur dioxide than any other stage, whereas Bovingdon (1935) finds that to hydrogen cyanide the egg is the least resistant stage of this insect.

In considering the relationship of metabolism to resistance at different times within one stage, discussion is again somewhat fruitless in the absence of reliable and apposite quantitative data. For various insect eggs, Fink (1925), Melvin (1928), Bodine (1929) and Burkholder (1934), have shown that there is an early "formative" period, which lasts a varying length of time according to the species of insect and is characterized by a low respiratory rate, and a later period, in which the metabolism increases up to the time of hatching. If this can be generally applied one would expect that the 0–1-day-old eggs of *Tribolium* would have a lower rate than eggs 3–4 days old, and according to Cotton's theory would be more resistant. Actually, as can be seen from Text-fig. 3, it was found that the older eggs are the more resistant to hydrogen cyanide. On the other hand, the resistance of the egg of the bed-bug to sulphur dioxide decreases with its age (Gough, 1938a). Uchida (1938) found that the susceptibility of eggs of *Chaetodacus dorsalis* Hend. to carbon disulphide increased as they developed.

The U-shaped curve mentioned by Wigglesworth (1934), which is characteristic of pupal metabolism, was obtained by Lindgren for pupae of *Tribolium*. He found that the susceptibility of pupae to chloropicrin and ethylene oxide also followed similar curves. The present author likewise found that recently formed pupae and those just about to emerge were much more susceptible than those in the middle period. Similar results were obtained by Uchida (1938) for pupae of *Chaetodacus dorsalis* Hend. and carbon disulphide.

Park (1936) found that females of *Tribolium confusum* have a higher respiratory rate than males. This may be correlated with the higher susceptibility to hydrogen cyanide of the females. Lindgren (1935)

records a considerable difference in the rate of metabolism of old adults with and without flour. As young adults did not exhibit this difference, he suggests that the adults do not feed for a few days after emergence. He found that the young adults were more resistant to carbon disulphide. The present writer found later (see § 6) that if older beetles were removed from flour about 1 hr. instead of the usual 20 min. before their fumigation they were much more resistant. Furthermore, several were stupefied at the end of the fumigation and recovered later like the 0–1-day-old adults. As the 0–1-day-old adults were never given the opportunity of feeding it was thought that this might account for their apparently different reaction. Accordingly, tests were carried out in the second apparatus, some on 0–1-day-old adults, which were supplied with flour as pupae, and others on 0–1-day-old adults which had no flour, as in the original experiment. The results were not significantly different as can be seen from the following figures expressed as mg./l. of hydrogen cyanide to give 50 % kill:

0–1-day-old adults emerged in flour   ... 0.629 ± 0.026
0–1-day-old adults without flour   ... 0.625 ± 0.051.

For several reasons these experiments are not quite comparable with the original ones, and also the males and females were not separated, as it appeared from other experiments that the two sexes did not differ greatly in resistance at this age.

The results described in § 6 suggest that under natural conditions there might not be such a great difference in resistance between young and old adults as would appear from the experiments described in this section. Further work is needed to elucidate this point.

Before leaving this question of the relationship of metabolism to resistance to poisons, attention should be drawn to a tabular summary of it in a recent paper by Busvine (1938).

5. INHERITANCE OF RESISTANCE TO HYDROGEN CYANIDE

Pupae, 2–3 days old, as can be seen from Text-fig. 4, exhibited a very wide range of variation in resistance. When the data were examined a difference was found between pupae obtained from different cultures.

These tests on different cultures were carried out at different times after a sufficient interval had elapsed for the results of tests carried out on pupae from one culture to be known. While the possible reasons for this variation are discussed in § 8, it seemed worth while investigating the possibility of inheritance of different degrees of resistance. Several

American workers have suggested that such a phenomenon would account for the increase in resistance to insecticides of certain scale insects (Melander, 1914; Quayle, 1922; Woglum, 1925). Boyce (1928) reported that after seven generations of breeding from individuals of *Drosophila melanogaster* which survived a highly toxic concentration of hydrogen cyanide, there was an average of 2 % more flies surviving than among the controls, when both were exposed to the same concentration of gas. He obtained a similar result for *Aphis gossypii*. In his experiments, insects which were highly susceptible were all killed, and offspring were only obtained from those flies which survived a high concentration of fumigant.
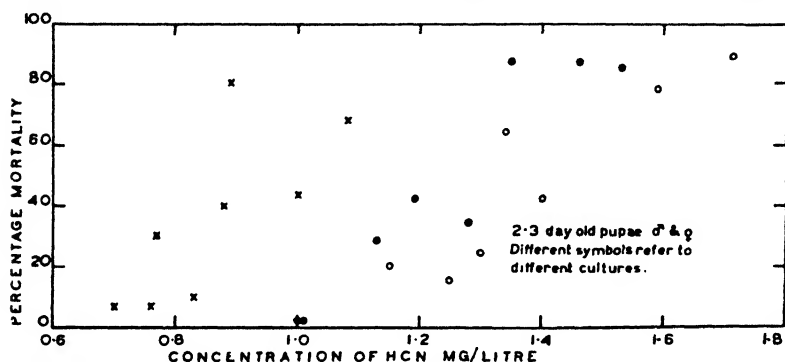
Text-fig. 4. Percentage mortality—concentration data for 2-3 days old pupae.

The experiments here described were so arranged that offspring were obtained from known beetles whose degree of resistance was then determined.

Eighty-four pairs of adult *Tribolium confusum* of the same age were made up into separate cultures and allowed to reproduce. The cultures were made up in 3 × 1 in. glass tubes, each about half-full of flour and covered with muslin. After 17 days the adults were removed and exposed for 1½ hr. to a concentration of hydrogen cyanide which was calculated to kill about 40 % of the beetles under the given conditions. Each pair of beetles was enclosed in a small separate cage, and twelve pairs were fumigated at a time. The cages were made by rolling up rectangles of iron wire gauze into cylinders about 5 mm. in diameter and 15 mm. long. One end was closed by pinching it with a pair of pliers and the beetles were dropped in. The other end was then closed in the same way. By stringing these cages on to a length of fine iron wire they could be kept in a known order and the difficulty of marking each cage dispensed with. Two

fumigations were performed daily until all the adults had been exposed to concentrations of hydrogen cyanide ranging from 0·63 to 0·66 mg./l. After fumigation, each pair was removed from its cage, examined, and transferred to a separate tube containing flour and kept in the constant temperature room. The following day they were examined for any further survivors. Where both the male and the female had survived this exposure, they were submitted, after an interval of a week, to a second, higher concentration of hydrogen cyanide. This was calculated to kill about 70 % of the beetles, which were then treated exactly as before.

The offspring of the eighty-four parents could then be divided into five lots:

(1) Both parents killed by the low concentration.

(2) Both parents survived the low concentration but were killed by the high one.

(3) Both parents survived both concentrations.

(4) One parent killed by the low concentration.

(5) Both parents survived the low concentration and one killed by the high one.

The last two groups were destroyed, leaving three groups whose parents had different degrees of resistance. There were five cultures in each group. Those in the first were labelled A, B, C, D, E; in the second, F, G, H, I, J; and in the third, K, L, M, N, O. Each of these is referred to as a family. As the first filial generation pupated, the pupae were removed at intervals and sorted into males and females. When these became adult, brother-sister cultures were made up daily until there were twelve of these from each of the original five cultures in each of the three groups, i.e. there were sixty pairs in each group. These were allowed to reproduce as before, and at the age of 17 days they were exposed to a certain concentration of hydrogen cyanide and the percentage kill in each family obtained. The same technique that was employed to deal with the parents was used also in this generation, except that they were only exposed to one concentration. In each fumigation, of about twelve pairs of beetles, there were representatives of each group and of as many families as possible. This mixing up of the members of different families instead of fumigating only the members of one family at one time eliminated or exposed any unforeseen factors which might have prejudiced the result. The mean concentrations for all fumigations in which there were representatives of any family have been calculated and are recorded in Table III. It will be seen that there is very little difference between these mean concentrations, although in the actual fumigations

the concentration varied from 0·57 to 0·62 mg./l. The results of any fumigations in which the concentration was outside this range, were neglected. Certain other experiments also had to be neglected as they gave an unexpectedly low kill. It has been mentioned that it was usual to perform two fumigations per day, one in the morning and the other in the afternoon. After several fumigations had been carried out it was noticed that the afternoon kill was considerably lower than the morning kill, the latter being about what was expected. Table II gives the results of morning (M.) and afternoon (A.) fumigations.

Table II

	Concentration of hydrogen cyanide mg./l.	No. fumigated	No. killed
M.	0·59	24	21
M.	0·50	22	14
A.	0·50	24	4
M.	0·59	24	13
A.	0·56	22	1
M.	0·60	24	12
A.	0·60	24	3

The only difference between the two lots was that, while in the morning the beetles were removed from their culture about half an hour before the commencement of the fumigation, the beetles for the afternoon fumigation were removed from their cultures about 1.0 p.m. and left in tubes without any flour during the lunch interval. They could then be tipped into their fumigation cages after lunch and the fumigation commenced almost immediately. This meant that about $1\frac{1}{2}$ hr. elapsed between the removal of the insects from flour and the commencement of the fumigation. This process was therefore reversed, so that in the morning there was a long interval, and in the afternoon a short one, with the following result:

	Concentration of hydrogen cyanide mg. l.	No. fumigated	No. killed
M.	0·59	24	0
A.	0·62	24	14

The short interval was thereupon taken as the standard and the remaining results were quite regular. This phenomenon is discussed, together with the results of some further experiments, in § 6 of this paper.

Table III gives the results of this experiment. The mean percentage kill and its standard errors are given for each group. The differences between groups 1 and 3 and 2 and 3 are significant but not the difference between groups 1 and 2.

Table III

Parent	No.	No. killed	%	Mean concentration mg./l.
Group 1. Both parents killed by a low concentration				
A	14	12	85·7	0·589
B	16	14	87·5	0·581
C	12	6	50·0	0·598
D	14	7	50·0	0·581
E	16	9	56·3	0·584

Mean % kill = 65·9 ± 8·68. Mean concentration = 0·587.

Parent	No.	No. killed	%	Mean concentration mg./l.
Group 2. Both parents survived low concentration and killed by high concentration				
F	10	4	40·0	0·592
G	10	8	80·0	0·592
H	14	9	64·3	0·587
I	12	7	58·3	0·590
J	16	8	50·0	0·592

Mean % kill = 58·5 ± 6·75. Mean concentration = 0·591.

Parent	No.	No. killed	%	Mean concentration mg./l.
Group 3. Both parents survived both concentrations				
K	18	4	22·2	0·587
L	14	3	21·4	0·587
M	14	8	57·1	0·589
N	20	7	35·0	0·576
O	16	5	31·3	0·597

Mean % kill = 33·4 ± 6·47. Mean concentration = 0·587.

As a further test, two fumigations were carried out in which about sixty adults from various families in groups 1 and 3 were exposed to the same concentrations of cyanide.

Concentration mg./l.	Group	No. fumigated	Percentage killed
0·64	1	55	82
0·64	3	60	43
0·59	1	52	54
0·59	3	60	37

The males and females were not sorted out in this test, and the high percentage kill in group 1 in the first test may be due to an unusually high proportion of females which are more susceptible than males.

Accurate work was difficult for two reasons:

(1) The great loss of gas during the course of the fumigation (see § 7).

(2) The difference in susceptibility of males and females being of the same order as the difference between the same sexes of two different groups.

On referring back to notes on the original fumigation of the parents, the same difference between morning and afternoon fumigations was observed. It is therefore clear that certain pairs of beetles were included

in group 2, which, had they been fumigated under exactly the same conditions as the others, might have come in groups 1, 4 or 5. It was then found that all the families in group 2 were descended from parents that had been fumigated in the afternoon. Group 2 was therefore artificial, and it is interesting to note that it was not significantly different from group 1. As the second series of fumigations, by which group 3 was defined, were all morning ones, this group was quite distinct from the other two. Group 2 was therefore eliminated from experiments on further generations. In order to reduce the numbers of insects to be handled, only the two most resistant, and the two most susceptible families were bred from. Those selected were A and B in group 1, and K and L in group 3. The pupae of the second filial generation of these families were removed from their cultures at intervals, and, as the adults emerged, fresh cultures of each family were set up about every other day. There were about forty to sixty beetles in each culture. When these adults had laid eggs and were from 12 to 14 days old, they were fumigated and dosage-mortality curves obtained from each family, using the same technique as has been described above for the different stages.

Experiments were not performed on the third, fourth, and fifth filial generations, but the stocks were maintained. Each family was kept down to about 1000 individuals in each generation. To avoid any unconscious selection in doing this, the insects which pupated in a given period were retained and the others destroyed. When the sixth filial generation was pupating, cultures were made up exactly as described above for the second filial generation. In the sixth and seventh filial generations about 100 beetles were available for each test and about ten tests were performed on each family. The second apparatus, which had two insect chambers, was used for these experiments. It was thus possible to carry out tests on two families simultaneously.

The results of all these experiments are shown in the concentration/ percentage mortality graphs in Text-fig. 9 and log concentration/probit graphs in Text-fig. 10. The limits of error of the concentrations required to give a 50 % kill have been calculated for the males and females, and these concentrations are shown in Table IV, and diagrammatically in Text-fig. 5. Only the 50 % points are shown for families A and K of the males, and K of the females in the second filial generation, as the data were such that a negative result was obtained for the factor $b^2 - t^2$ (Vb) of Bliss's equation, i.e. the slope of the regression line does not differ significantly from the horizontal.

The high error in the estimate of the 50 % kill of females of the B and

37-2

L families of the F_2 generation is due to the fact that most of the kills were over 50 % and that the slope of the regression line was very low. For reasons described in § 8 the result of tests on females of the F_6 generation in family A are not given here.

Table IV. *Summary of data of inheritance experiments*

Family	Generation	n	χ^2	50 % kill mg./l.
			Males	
A	F_2	3	7·2158	0·592
B	F_2	5	9·6656	0·426 ± 0·047
K	F_2	3	5·9311	0·617
L	F_2	4	1·2827	0·782 ± 0·049
A	F_6	6	5·3119	0·846 ± 0·034
B	F_6	8	12·0418	0·780 ± 0·054
K	F_6	7	· 18·7751 (S.)	0·919 ± 0·024
L	F_6	8	7·1134	1·031 ± 0·036
A	F_7	16	74·9525 (S.)	0·696 ± 0·017
B	F_7	10	55·6249 (S.)	0·679 ± 0·028
K	F_7	9	.107·5925 (S.)	0·867 ± 0·042
L	F_7	10	10·6023	0·840 ± 0·019
			Females	
A	F_2	3	0·4548	0·502 ± 0·024
B	F_2	6	18·2383 (S.)	0·356 ± 0·197
K	F_2	3	4·4834	0·384
L	F_2	4	2·5454	0·553 ± 0·186
A	F_6	—	—	—
B	F_6	8	31·0979 (S.)	0·650 ± 0·025
K	F_6	7	5·2966	0·851 ± 0·034
L	F_6	8	69·6269 (S.)	9·845 ± 0·041
A	F_7	16	39·3113 (S.)	0·649 ± 0·027
B	F_7	10	107·4017 (S.)	0·678 ± 0·036
K	F_7	9	10·3129	0·781 ± 0·034
L	F_7	10	. 14·9025	0·829 ± 0·027

Where the data depart significantly from the straight line that has been fitted to them, the value of χ^2 is followed by S. The limits of error above and below the 50 % kill value are not necessarily the same, but for convenience the larger error is given.

It will be seen from Text-fig. 5 that there is a considerable difference in resistance from generation to generation. The different apparatus may account for the difference between the second and sixth filial generations; as has been explained above, there was a large surface of mercury exposed in the first apparatus and the vapour was found to be highly toxic to insect eggs. Eggs of both *Tribolium* and the bed-bug were killed by exposure to mercury vapour for less than 24 hr. at 27 and 23° C. respectively, whereas other stages of these insects were unaffected under the same conditions. It would be surprising if the greater surface of mercury exposed had such a marked additional toxic effect upon adults which were apparently unaffected after a week's exposure to mercury

vapour. Other references to this have been recently recorded by Gough (1938b).

There seemed to be no obvious reason for the difference between the sixth and seventh filial generations. Shepard *et al.* (1937) were able to reproduce after an interval of time their original results of fumigation experiments on *Tribolium*. Their data were also in agreement with those of other workers. On the other hand, with other fumigants and test insects their results were more variable. Tattersfield & Martin (1935) have stressed the importance of carrying out comparative toxicity tests at not too widely separated intervals of time, though they were more particularly concerned with plant feeding insects.
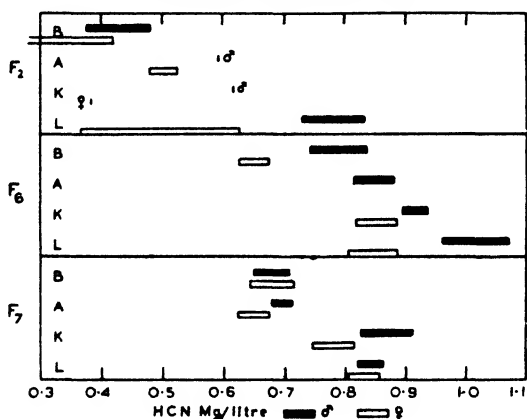
Text-fig. 5. Limits of error of concentrations of hydrogen cyanide required to give a 50 % kill of different families of beetles (B, A, K, L) in different generations (F_2, F_6, F_7).

Even more surprising is the difference in the relative resistance of males and females in the different generations. It is quite evident from either Text-fig. 10 or Table IV that in the second filial generation the females are much more susceptible than the males, whereas in the seventh filial generation, the resistance of the two sexes is about the same. It is possible that as normally the female appears to be more susceptible than the male, the original fumigation of the parents tended to select the more resistant females. If this were so, it is difficult to understand why this increase in resistance of the females was not observed in the second filial generation.

Throughout the series there is, however, a distinct significant difference between the males of the resistant and of the susceptible groups, especially between families B and L. In both the second and the

sixth filial generations the order of resistance is the same, i.e. B, A, K, L, and in both generations the A and K families are very close together, although in the seventh filial generation these two families are as widely different as the other two. In the females the difference between the two groups is significant in the sixth and seventh generations, though in the second generation the 50 % kills for A are higher, and for K lower than would have been expected. This evidence of the existence of the inheritance of resistance towards certain poisons obviously has a very important bearing on all chemical methods of insect control. It is, perhaps, likely to be of less importance in plant protection, where the entomologist is more concerned in reducing the numbers of a pest to a certain economic limit, than in the fumigation of stored products, where a complete kill is the aim. In the former type of control vast numbers of insects escape for purely accidental reasons and not necessarily because they are more resistant. These insects might keep the resistance of the insect population to a given poison at its normal level. In a well-conducted fumigation the most likely reason for insects surviving is that they are highly resistant to the fumigant, and if these insects alone are allowed to reinfect the same product the population derived from them might have a considerably higher level of resistance than normal. It would be interesting to know if an insect which is highly resistant to a certain poison in one stage, is also more resistant in other stages, and also if it is more resistant to other poisons.

Correlation of resistance with other characters

Having obtained families of beetles with significantly differing degrees of resistance to hydrogen cyanide an attempt was made to correlate this with other characters. There was no superficial morphological difference, nor was there much difference in the rates of development as measured by length of life cycle, though family K appeared to take a few days longer than any of the others. This slight lengthening of life cycle apparently occurred in the larval stage.

There did not seem to be any simple relationship between body size, as measured by live weight, and resistance:

Wt. of 100 beetles, F_7			
B (most susceptible to cyanide)	A	K	L (most resistant to cyanide)
0·2114 g.	0·1872 g.	0·1939 g.	0·2114 g.

Mr G. V. B. Herford carried out respiration experiments on different families in a modified Barcroft respirometer. The following are the results

expressed as the oxygen uptake per mg. live weight of beetle in c.mm./hr. at 27° C. 100 beetles were used in each experiment:

B (most susceptible to cyanide)	A	K	L (most resistant to cyanide)
2·163	2·422	1·925	2·803

If these experiments can be considered representative they suggest that a difference in rate of metabolism between individuals is not necessarily correlated with a difference of resistance, as L has the highest respiratory rate and yet is the most resistant family. B has a lower respiratory rate than any of the others except K, and yet is the most susceptible to hydrogen cyanide. As these respiration experiments were not repeated and other respiration measurements showed considerable variation, they cannot be taken as evidence against the validity of Cotton's theory. It must, however, be regarded as possible that respiratory rate is not necessarily the only factor which determines resistance, even within one stage of an insect. The relationship of resistance to metabolism between different individuals of one stage must, with our present knowledge at any rate, be considered separately from the relationship of resistance to metabolism between the different stages of one insect.

6. Effect on resistance to hydrogen cyanide of the time elapsing between removal of the beetles from flour and their fumigation

It was not possible to investigate this question as fully as was desired, so that the results are only comparative and qualitative.

A number of cultures of adult beetles were made up and left in the constant temperature and humidity room. After some days the beetles were removed from the flour at certain specified times to empty jars. This operation was carried out in the constant temperature room. The beetles were then exposed to the same concentration of hydrogen cyanide for $1\frac{1}{2}$ hr. and afterwards the males and females sorted and the percentage kill obtained. The results are shown in Table V.

It will be seen from these tables that there is considerable variation in the results of the three experiments. There was some difference in the age of the three lots. Also the beetles in Exp. 3 were left in flour for a much longer period before the experiment was carried out. In spite of the variation, certain facts emerge.

Table V

No. of hours previously removed from flour	Concentration mg./l.	Males				Females			
		No. fumigated	Recoveries 1st day after fumigation	2nd day after fumigation	% kill	No. fumigated	Recoveries 1st day after fumigation	2nd day after fumigation	% kill
			Experiment 1						
24	1·07	40	2	1	63	49	9	4	41
6	1·06	32	1	0	65	61	11	8	25
3	1·07	50	1	1	78	66	9	1	42
1	1·06	36	0	0	80	52	2	5	65
½	1·05	40	0	0	82	52	2	6	71
0	1·05	36	0	0	86	54	1	0	89
			Experiment 2						
20	1·01	79	4	4	67	44	9	8	41
15	1·01	76	8	3	74	59	10	7	48
6	0·99	75	2	0	59	73	9	7	40
3	0·99	70	2	2	64	84	5	1	31
1	0·97	70	3	1	61	61	3	9	54
0	0·97	64	4	1	69	74	2	1	92
			Experiment 3						
12	1·66	54	1	5	61	61	1	7	36
8	1·66	55	2	7	55	57	0	7	37
8	1·73	50	3	0	54	59	0	1	37
4	1·79	47	1	0	74	64	0	2	48
2	1·81	50	1	1	68	48	1	0	44
0	1·79	45	0	0	82	49	1	0	76

(1) The males are less affected by early removal from flour than are the females. The following are the lowest and highest kills of the two sexes in each experiment:

Exp.	Males %	Females %
1	63–86	25–89
2	59–74	31–92
3	54–82	34–76

(2) It seems that there are rather more recoveries of apparently dead beetles afterwards among the females than among the males. There are also more recoveries in both sexes in those insects which have been out of flour for the longer periods. This suggests that lack of flour tends to produce a greater degree of stupefaction. This might account for the greater resistance of these beetles by a "protective stupefaction" effect as described by Pratt *et al.* (1931).

There are several possible explanations.

(i) *Effect of mechanical shock*

The actual mechanical shock of being removed from flour might affect the insects merely by making them more active, and possibly more susceptible. This seemed unlikely for two reasons:

(*a*) All beetles were disturbed to some extent during transference from the empty jar to the fumigation cage, and the introduction of that into the insect chamber.

(*b*) If this theory were correct it might be supposed that the increase in resistance with increasing time out of flour (up to several hours in each experiment) was accounted for by the beetles taking up to that number of hours to settle down. Actually, as far as could be observed, they settled

Table VI

	No. of hours previously removed from flour		% kill	
			Males	Females
(1)	8	Not shaken	54	37
	8	Shaken	67	34
	2	Not shaken	68	44
	2	Shaken	84	61
	0	—	82	76
(2)	2	Not shaken	30	49
	2	Shaken	56	53
(3)	2	Not shaken	53	44
	2	Shaken	41	43

down within about 15 min. of being removed. It is possible, however, that the effect of activity lasts for a greater period than the activity itself.

Experiments were carried out in which two lots of insects were removed from flour at a certain time and placed in their fumigation cages. Immediately before fumigation the one lot was treated exactly as it would have been had it just been removed from flour, i.e. the beetles were shaken in a sieve, removed from this by tapping on to a piece of paper, brushed into a tube with a fine brush, and finally tipped into the cage again and fumigated with the other lot which was placed at the same time as gently as possible in the fumigation chamber. The results are shown in Table VI.

Though, once again, these results are conflicting it seems that this is at least one of the factors concerned. It may operate through the production of a volatile substance emitted by the adults, under certain conditions (see § 7).

(ii) *Effect of contact with flour*

It is possible that mere contact with flour has some effect on the insects, perhaps on the tone of the muscles, or the respiratory rate or the degree to which the spiracles are opened. Though no experiments were performed, this possibility might be tested by placing the beetles in some non-toxic and inert substance similar in texture to flour, and seeing if removal from that substance at various times affected their resistance.

(iii) *Amount of food in the gut*

If the insect is feeding practically all the time, the amount of food present in some part of the gut may affect its resistance directly or indirectly. It seems unlikely that such a short period as an hour would have so great an effect as was observed but, on the other hand, it is interesting to compare the similarity in reaction to hydrogen cyanide of 0–1-day-old adults and older adults which have been removed from flour some time previously. As has been mentioned, Lindgren (1935) found a great difference between the rate of metabolism of old adults in flour and out of flour. Young adults, by which he meant those less than 4 days old, did not exhibit this difference, and had about the same rate of metabolism as older adults in flour. Lindgren suggests that the young adults do not feed for some days after emergence. If this could definitely be demonstrated it would at least suggest that the amount of food in the gut had some influence on resistance.

Craufurd-Benson (1938) obtained more uniform results in toxicity tests of Derris preparations to *Ahasverus advena* Waltl. when the insect was removed from its food medium 24 hr. before test, than when insects freshly removed were tested. He suggests that this period of starvation ensures uniformity of metabolic processes.

7. EMANATION OF A SELF-TOXIC AND VOLATILE SUBSTANCE BY THE ADULTS

(i) *Emanation of a self-toxic substance*

During early tests a muslin bag was used to contain the insects. This was not very satisfactory as it absorbed hydrogen cyanide, and it was not easy to insert and extract insects, especially the adults. Another type of container used was a short length of glass tubing about 1 cm. long and 0·5 cm. internal diameter. This was closed at each end with bolting silk secured with fine iron wire. At the end of the first test of adult

beetles in this type of container all the controls were found to be dead or moribund. The latter would react to stimulation by twitching and some were sufficiently lively to attempt to walk when placed right side up but had difficulty in co-ordinating their movements. A series of experiments was then carried out with various numbers of adults of different ages enclosed in this container. All these experiments were carried out in the constant temperature room (see Table VII).

Table VII

Sex	Whether mated (M.) or virgin (V.)	Age weeks	No. of hours in container	No. of beetles	No. normal	No. twitching	No. motionless	% affected
Male	M.	2	19	42	42	0	0	0
Male	M.	2	19	40	40	0	0	0
Female	M.	2	19	41	41	0	0	0
Female	M.	2	19	47	47	0	0	0
Male and female	M.	4	2	60	60	0	0	0
Male and female	M.	4	16	55	55	0	0	0
Male	M.	5	2	73	11	9	53	85
Female	M.	5	2	59	12	39	8	80
Male	V.	6	2	61	53	4	4	13
Male	V.	6	16	60	55	2	0	3
Male	V.	6	16	49	48	1	0	2
Female	V.	6	2	60	0	4	56	100
Female	V.	6	2	30	2	6	22	93
Female	V.	6	2	15	0	11	4	100
Female	V.	6	2	5	0	0	0	0
Female	V.	6	20	5	4	1	0	—

It appeared from these experiments that from about the age of 4–5 weeks the adults, under certain conditions, emitted some substance which was self-toxic and, furthermore, that while mated adults of both sexes produced this substance, virgin females produced more than virgin males. That the higher mortality among these females was not due to greater susceptibility was shown by the fact that males placed in contact with them were equally affected.

(ii) *Volatility of the self-toxic substance*

The following experiments suggested that this self-toxic substance was volatile:

(1) Two containers were placed in line with one another and were only separated by muslin. Adults which produced this emanation were placed on one side and adults which apparently did not produce it on the other side. The latter were affected, showing that the emanation had diffused from one container to the other. Similar results were obtained

if the second type of adults were suspended in a muslin bag over a number of the emanation-producing adults, all in a closed tube.

(2) Beetles placed in a container, through which a slow stream of air was drawn, were unaffected, suggesting that the substance was removable as a gas by convection. Under such conditions, however, they might not be stimulated to produce the substance.

(3) Beetles placed in a muslin bag were not affected. Under these conditions there is sufficient opportunity for the substance to escape by diffusion and convection.

As some experiments suggested that this substance was produced in much greater amounts by virgin females than by any other type of adult, it was thought that it might be an aphrodisiac. An experiment was performed in which a number of males and a number of females were enclosed at the two ends of the branches of a Y tube. Virgin males were then admitted at the base of the Y. Practically equal numbers went in each direction.

Effect of high humidity and high concentrations of carbon dioxide.

In order to eliminate these two obvious possibilities the following experiments were carried out. In the first, small numbers of beetles were placed in a tube over water. None of these was affected. In the second, a tube with a large number of beetles was surrounded by soda lime: forty-eight out of fifty of these beetles were affected within 2 hr. It was noticed that the soda lime near each muslin-covered end of the tube had turned a dull green. It was then found that if a small flake of sodium or potassium hydroxide were held over a number of adult beetles a green coloration developed on the surface of the flake. The speed with which this occurred, depended on the number of insects and the period during which they had been enclosed. If the sodium hydroxide was left for several hours near the beetles it ultimately turned a rusty brown colour.

Whether this substance was actually given off as a gas or, as seems more likely, as a liquid secretion which subsequently volatilized is not known. It may be emitted by special glands or might even be given off from the faeces. In tubes in which the insects had been kept the muslin and the sides of the tube were usually discoloured.

(iii) *Absorption of hydrogen cyanide by a volatile substance evolved by the adults*

It has been explained that a gas sample was taken at the beginning and end of each test. Usually there was a slight loss of gas during the course of the test due to absorption by the insects, their container, stop-

cock grease, etc. In a series of twenty tests on eggs, larvae, pupae and freshly emerged adults, the average loss per test was 0·037 mg./l., ranging from 0 to 0·06 mg./l. A series of tests on 28-day-old adults then gave the following losses in order:

0·07, 0·10, 0·09, 0·16, 0·18, 0·21 mg./l.

A test was then performed without any insects and there was a loss of 0·18 mg./l. Flask X was then isolated, and gave a loss of 0·05 mg./l. It was thought possible that some spray from the solution of potassium hydroxide, which was used for conditioning the air, might have been drawn into the apparatus, so this was dismantled and cleaned. The mercury was washed with dilute nitric acid followed by distilled water. All rubber connexions were renewed and all glass parts cleaned with nitric cleaning mixture. The apparatus was then reassembled. Sulphuric acid solution was used for conditioning the air, and an extra spray trap was also inserted. Forty-six tests were then carried out on eggs, larvae and pupae and the loss of hydrogen cyanide never exceeded 0·04 mg./l., the average being just over 0·02 mg./l. Then eight tests on adults were performed and the average loss was 0·07, ranging from 0·06 to 0·16 mg./l. Only fairly small numbers of adults were used in each of these tests. Nine tests on stages other than adults then gave an average loss of 0·08 mg./l., and the next eleven tests, also on other stages, gave an average loss of 0·04 ranging from 0·03 to 0·07 mg./l. Then eight more lots of adults were fumigated with the following losses: 0·12, 0·16, 0·21, 0·21, 0·17, 0·26, 0·30, 0·30 mg./l. The last losses each represented about 40 % of the original concentration of hydrogen cyanide in those tests. The apparatus was then cleaned out again and similar results obtained, i.e. with all stages but adults the loss was practically negligible, but when adults were used the loss became very high. Moreover, the effect was cumulative, that is, the loss of cyanide increased each time adults were used, and was noticeable for some time after adults had been used. This suggested that the adults give off some volatile substance which combines loosely with hydrogen cyanide under certain conditions. That the loss is due merely to a greater absorption by the adults is ruled out by the fact that freshly emerged adults do not cause it, and by the fact that it is residual and occurs also after the insects have been removed. As the apparatus was evacuated twice between each test, it must be assumed that this substance either condenses out again on the internal surfaces of the apparatus or, very improbably, combines in some way with either the glass or the mercury. As the lower ends of the large vessels X and Y were

possibly at a slightly lower temperature than the upper ends it may be that condensation tended to occur there. Probably at the reduced pressure during evacuation the compound containing hydrogen cyanide dissociated and the hydrogen cyanide itself was removed, leaving the substance on the walls of the glass vessels. It is remarkable that the repeated evacuation failed to vaporize it. It was also unaffected by a high concentration of cyanide. In order to make use of tests on adults an arbitrary concentration had to be selected. As the concentration, taken every 20 min., plotted against time was found to give a straight line, it was assumed that the mean of the first and final gas samples would give a fairly good idea of the effective concentration.

How far this substance can be identified with the one which was self-toxic it is difficult to say. In all the tests on adults, wire-gauze cages were used and the controls were quite unaffected. It may perhaps be assumed that this substance is being emitted all the time, and in the muslin-capped tubes it was not removed sufficiently quickly by diffusion. 14-day-old adults were, however, unaffected by being enclosed in the muslin-capped tubes, but they certainly caused as great a loss of cyanide as the older adults. The difficulty of carrying out analytical tests on this substance is emphasized by the fact that the greatest loss of cyanide was about 0·3 mg./l. or a total loss of about 0·00165 g. Presumably the substance concerned would be present in an amount of the same order.

(iv) *Other references to emanations of* Tribolium

These emanations may also be associated with Park's (1935) work on the conditioning of flour by *Tribolium*, and possibly with the peculiar smell of this insect. Chapman (1926) mentions that a gas is given off by adults of *Tribolium* when irritated. When larvae and pupae were enclosed with a number of beetles which had been irritated, about 10 % of these larvae and pupae gave rise to monstrosities. Mature larvae emerged with a combination of larval and pupal structures and pupae gave rise to deformed adults. Chapman associates these abnormalities with other reports of abnormal metamorphosis in insects.

It seems, therefore, that a study of this emanation of *Tribolium* might lead to very interesting and important results, and the brief observations described here may suggest lines along which the chemical constitution of this emanation may be investigated.

8. THE DOSAGE-MORTALITY CURVE

Text-figs. 6–10 are graphs expressing the relationship between concentration of hydrogen cyanide and percentage mortality, or log concentration and mortality converted to probits. As the points were so scattered no attempt was made to fit the concentration-mortality curves. Only the graph of 0–1-day-old eggs in Text-fig. 6 shows anything like a smooth curve and the histogram on the same graph has been derived from this curve by plotting differences in percentage kill for regular dose
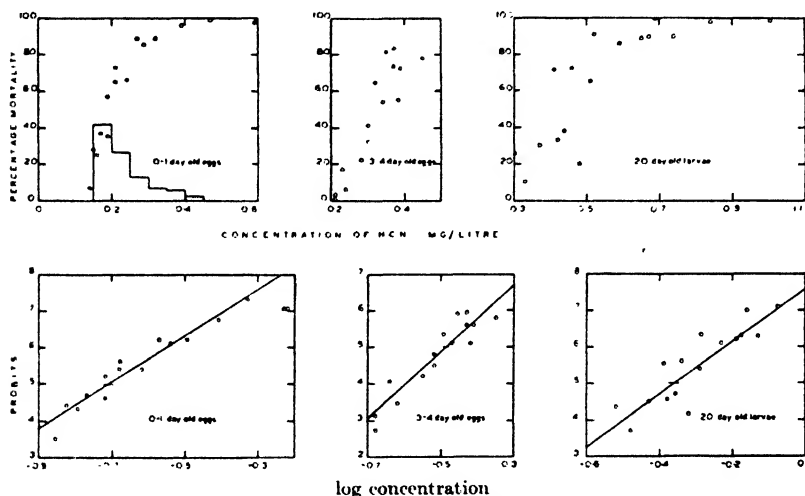
Text-fig. 6. Dosage-mortality graphs for eggs and larvae.

intervals (0·05 mg./l. here) against the mid-point of that dose interval. This method is described by Trevan (1927). The histogram exhibits the usual type of asymmetry though it is exceptionally well marked. This is partly due to the allowance for the failure of a certain percentage of the controls to hatch, and possibly to the great error in the estimation of hydrogen cyanide at the very low concentrations.

As might be expected from the irregularity of these curves, the values for χ^2 of the regression line obtained by Bliss's method are all very high. Out of fifteen regression lines of different stages of *Tribolium*, twelve diverged significantly from a straight line. It is interesting to note that the remaining three were experiments in which fewer tests were carried out. In the inheritance experiments nine out of twenty-four regression lines diverged significantly from the straight line fitted to them.

Craufurd-Benson (1938) obtained erratic results in toxicity tests of Derris preparations to *Ahasverus advena* Waltl. until he standardized his technique very rigidly. In the present work, as temperature and humidity, length of exposure, gas concentration and circulation, were all controlled it is difficult to account for the great variation observed in the results. The following are possible suggestions:

(1) The typical dosage-mortality curve is not necessarily applicable to the reaction of all organisms to all poisons. In view of the frequency with which this type of curve is obtained when the percentage of a number
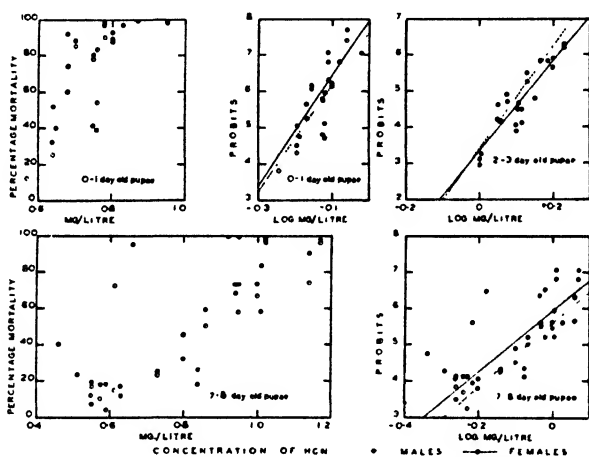
Text-fig. 7. Dosage-mortality graphs for pupae.

of organisms reacting in a certain way to a certain stimulus, is plotted against a function of the magnitude of that stimulus, this suggestion is scarcely acceptable. Strand (1930), and many other workers since, have used *Tribolium confusum* as a test insect and have obtained normal curves. Bliss (1935a) has analysed Strand's data and found them consistent with a straight regression line, when converted to probits and log concentrations.

It is also a difficult matter to decide whether or not to include points which are nowhere near any others, such as the two in the top left-hand corner of the graph of 7–8-day-old pupae in Text-fig. 7. Are these accounted for by random sampling, or is it justifiable to assume that something went wrong in those particular tests? Trevan (1927) has stated: "One thing should be insisted on, however, with great force, and that is,

that unexpected or 'discrepant' results must not be eliminated from the final average, except on the clearest evidence of a mistake in the technique." This rule has been followed in the present work. In the experiment on 2–3-day-old pupae (Text-fig. 4), it was thought reasonable to consider the tests on cultures indicated by a cross, distinct from the others, and only the latter were used when the results were treated with Bliss's method. On the other hand, odd points as those mentioned above have always been included in the calculations.
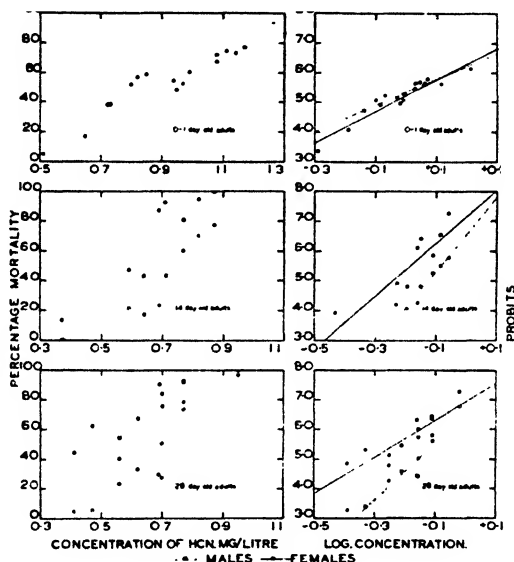
Text-fig. 8. Dosage-mortality graphs for adults.

Had the majority of the tests given consistent results it might have been reasonable to have neglected the results of "discrepant" tests, but under the circumstances it was thought best to include them all.

Several of the curves, especially those for larvae (Text-fig. 6), for 7–8-day-old pupae (Text-fig. 7) and certain of the curves shown in Text-figs. 9 and 10 exhibit, in places, a tendency to show no increase in mortality with an increase in dose. This might cast doubt on the accuracy of the estimation of the gas concentration, but as the two samples taken in each test were almost invariably similar, it may fairly be assumed that the method of gas sampling was satisfactory. If, to account for these "step-curves", one assumes that there are well-defined degrees of resistance to

hydrogen cyanide in *Tribolium*, it would be expected that after in-breeding for several generations as had been done, this "step" effect would disappear. The curves for the A, B, and K families in the seventh filial generation (Text-figs. 9, 10) show that this is not so. In the seventh filial generation of family A this "step" effect is very marked. An exceptionally large number of tests were performed in this experiment
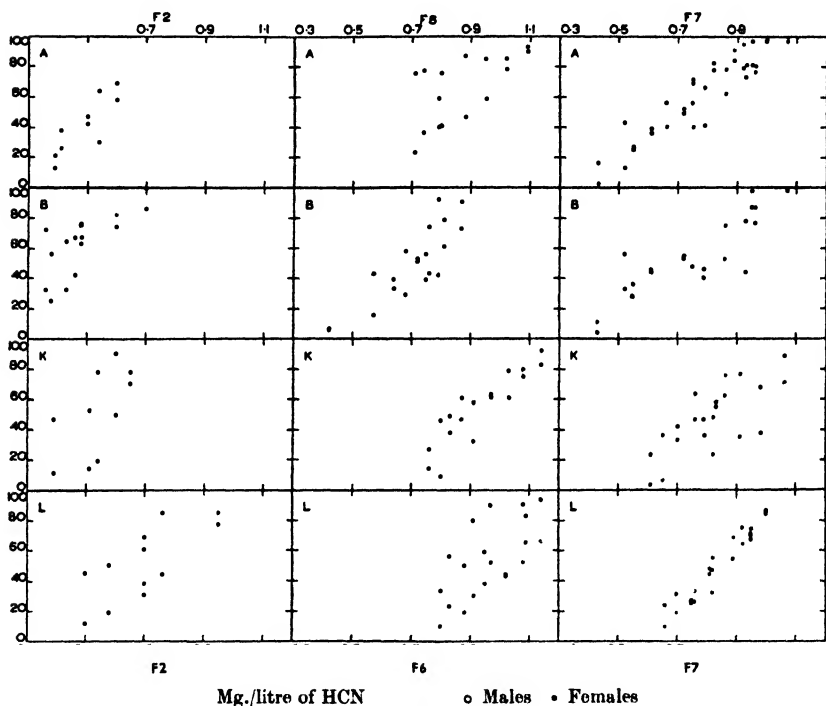
Text-fig. 9. Percentage mortality-concentration graphs for 3 generations F_2, F_6 and F_7 of 4 different families A, B, K, L of *Tribolium confusum*.

since in the previous generation all the points for females, with one ex-ception, lay in the 80–90 % region, whereas the points for the males at the same concentrations ranged from 20 % upwards. As a number of dead beetles had been found in these cultures it was thought that the females might have been suffering from some disease which killed some of the beetles and lowered the resistance to hydrogen cyanide of the others. The dead insects were therefore examined, and it was found that they were mostly males. Hence, it was desired to see if a similar curve for

females would be obtained in the next generation. Though this was not found, there was a tendency for the curve to flatten out in the 80 % kill region. In the curve for family B in this generation there appears to be a horizontal portion about the 50 % kill region. Without performing very large numbers of tests under very carefully controlled conditions it would be difficult to decide whether the peculiar shape of these curves was real or not. The fact that the Slough stocks of *T. confusum*, from which the author's experimental insects were derived, had been inbred for many
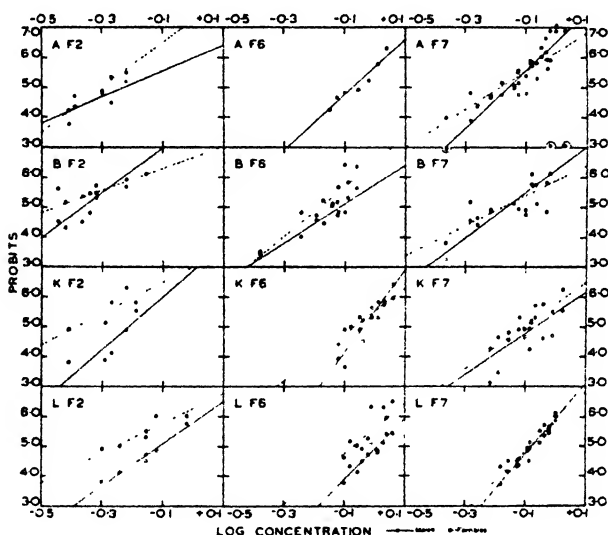
Text-fig. 10. Probit-log concentration graphs for 3 generations F_2, F_6 and F_7 of 4 different families A, B, K, L of *Tribolium confusum*.

years and must have been fairly homogeneous, suggests that one must look elsewhere for an explanation of these curves.

(2) Trevan (1927) came to the conclusion that a group of about thirty animals was near the optimum size for the determination of an average lethal dose, and a significant increase in accuracy above that given by this group number is only obtained by the use of greatly increasing numbers of animals. As, nearly always, larger numbers than this were used in each test, it seems unlikely that insufficient numbers of insects were used to give accurate results.

(3) Perhaps the most likely explanation of the variable results is the short exposure period of 1½ hr. Most other workers have used longer

periods, 5 hr. being most usual. This was tried in the first place, but the great susceptibility of *Tribolium* to hydrogen cyanide made dosing difficult and estimation of the low gas concentrations necessary was very inaccurate. Furthermore, it was desirable to be able to perform two or three tests per day.

In a short exposure the two following factors would probably have a much greater influence on the final reaction of an insect to a poison than in a longer exposure.

(*a*) *The effect of handling.* It is practically impossible to carry out such tests as these without handling the insect to some extent. The experiments described in § 6 show that the treatment *Tribolium* receives before being fumigated greatly affects its reaction. It seems unlikely, however, that disturbance due to handling would affect eggs and pupae as much as larvae and adults, whereas, in fact, all stages exhibited equally great variability.

(*b*) *Initial reaction of the insect when exposed to the gas.* This reaction will vary with the insect and the fumigant and the concentration of the fumigant. When introduced suddenly into a fumigant an insect may be stupefied almost immediately, it may be stimulated to greater activity, it may close its spiracles or open them wider, or other less obvious but equally important processes may occur. After this initial reaction, the insect may reach a more or less steady state, and it may be that the greater the length of this steady period, the less variable will the results be. The results of Pratt *et al.* (1931), on the "protective stupefaction" of certain scale insects when exposed to sublethal concentrations of hydrogen cyanide, indicate that the initial reaction of an insect to a fumigant is of great importance.

9. SUMMARY

1. Two types of apparatus for exposing insects to known concentrations of toxic gases, under controlled conditions, were constructed and are described.

2. The relative resistance of different stages of the flour beetle, *Tribolium confusum* Duv., to hydrogen cyanide was determined, the results being treated by Bliss's method. The order of resistance was pupa (most resistant), adult, larva, egg. The difference in resistance at different ages was found for the pupa, adult and egg. The application to all these results of Cotton's theory of the relation of rate of metabolism and susceptibility to fumigants is discussed. It is considered that this theory might hold for stages other than the egg.

3. A series of experiments was carried out to discover whether individual resistance to hydrogen cyanide was inherited. It was found that the offspring of resistant individuals were significantly more resistant than the offspring of susceptible individuals, and that this difference was maintained over several generations.

4. A considerable increase in resistance was found to occur if adult beetles were removed from their flour about an hour, instead of immediately, prior to their fumigation. The reason for this increase was not determined, but various suggestions to account for it are discussed.

5. It was noted that under certain conditions, adult beetles emitted a volatile substance which was self-toxic, and also appeared to react with cyanide.

6. The dosage-mortality curves for all the experiments are discussed and possible reasons for their apparent abnormality put forward.

7. The experiments here described, which must only be regarded as preliminary, emphasize

(i) The complexity of the problems arising in the study of the resistance of living organisms to toxic substances.

(ii) The need for carrying out such experiments under as natural conditions as possible.

(iii) The importance of a careful analysis of factors which affect the resistance of the organism.

REFERENCES

BLISS, C. I. (1935a). The calculation of the dosage-mortality curve. *Ann. appl. Biol.* **22**, 134.

—— (1935b). The comparison of dosage-mortality data. *Ann. appl. Biol.* **22**, 307.

BODINE, J. H. (1929). Factors influencing the rate of respiratory metabolism of a developing egg (Orthoptera). *Physiol. Zoöl.* **2**, 459.

BOVINGDON, H. H. S. (1934). An improved laboratory apparatus for fumigation experiments. *Ann. appl. Biol.* **21**, 704.

—— (1935). The bed-bug (*Cimex lectularius* L.), Fam. Cimicidae. *Rochester Nat.* N.S., **1**, 3.

BOYCE, A. M. (1928). Studies on the resistance of certain insects to hydrocyanic acid. *J. econ. Ent.* **21**, 715.

BUSVINE, J. R. (1938). The toxicity of ethylene oxide to *Calandra oryzae, C. granaria, Tribolium castaneum* and *Cimex lectularius*. *Ann. appl. Biol.* **25**, 605.

BURKHOLDER, J. R. (1934). A quantitative study of respiratory metabolism in single developing eggs (Orthoptera). *Physiol. Zoöl.* **7**, 247.

CHAPMAN, R. N. (1926). Inhibiting the process of metamorphosis in the confused flour beetle. *J. exp. Zool.* **45**, 293.

COTTON, R. T. (1932). The relation of respiratory metabolism of insects to their susceptibility to fumigants. *J. econ. Ent.* **25**, 1088.

CRAUFURD-BENSON, H. J. (1938). A method for testing liquid contact insecticides. *Bull. ent. Res.* **29**, 41.

FINK, D. E. (1925). Metabolism during embryonic and metamorphic development of insects. *J. gen. Physiol.* **7**, 527.

FISHER, R. A. (1932). *Statistical Methods for Research Workers*. Oliver and Boyd.

GOUGH, H. C. (1938a). Use of sulphur dioxide against the bed-bug. *Nature, Lond.*, **141**, 164.

—— (1938b). Toxicity of mercury vapour to insects. *Nature, Lond.*, **141**, 922.

HAMLIN, J. C. & REED, W. D. (1927). Insect revival after fumigation. *J. econ. Ent.* **20**, 400.

KNIGHT, H. (1925). Factors affecting efficiency in fumigation with hydrocyanic acid. *Hilgardia*, **1**, 35.

LINDGREN, D. L. (1935). The respiration of insects in relation to the heating and fumigation of grain. *Tech. Bull. Minn. agric. exp. Sta.* **109**.

LUBATTI, O. F. (1935). Determination of fumigants. III. Micro-determination of ethylene oxide and hydrogen cyanide. *J. Soc. chem. Ind., Lond.*, **54**, 424.

MELANDER, A. L. (1914). Can insects become resistant to sprays? *J. econ. Ent.* **7**, 167.

MELDRUM, N. U. (1933). *Cellular Respiration*. Methuen.

MELVIN, R. (1928). Oxygen consumption of insect eggs. *Biol. Bull. Wood's Hole*, **55**, 135.

PARK, T. (1935). Studies in population physiology. IV. Some physiological effects of conditioned flour on *Tribolium confusum* Duval and its populations. *Physiol. Zoöl.* **8**, 91.

—— (1936). Studies in population physiology. V. The oxygen consumption of the flour beetle *Tribolium confusum* Duval. *J. cell. comp. Physiol.* **7**, 313.

POTTER, C. (1937). A biological study of the fumigation of empty warehouses with hydrogen cyanide and ethylene oxide. *Ann. appl. Biol.* **24**, 415.

PRATT, F. S., SWAIN, A. F. & ELDRED, D. N. (1931). A study of fumigation problems: "Protective Stupefaction," its application and limitations. *J. econ. Ent.* **24**, 1041.

QUAYLE, H. J. (1922). Resistance of certain scale insects in certain localities to hydrocyanic acid fumigation. *J. econ. Ent.* **15**, 400.

SHEPARD, H. H., LINDGREN, D. L. & THOMAS, E. L. (1937). The relative toxicity of insect fumigants. *Tech. Bull. Minn. agr. exp. Sta.* **120**.

STRAND, E. D. (1930). Measuring the toxicity of insect fumigants. *Industr. Engng Chem.* (Anal. Ed.), **2**, 4.

TATTERSFIELD, F. & MARTIN, J. T. (1935). The problem of the evaluation of rotenone-containing plants. I. *Derris elliptica* and *Derris malaccensis*. *Ann. appl. Biol.* **22**, 578.

TREVAN, J. W. (1927). The error of determination of toxicity. *Proc. roy. Soc. B.*, **101**, 483..
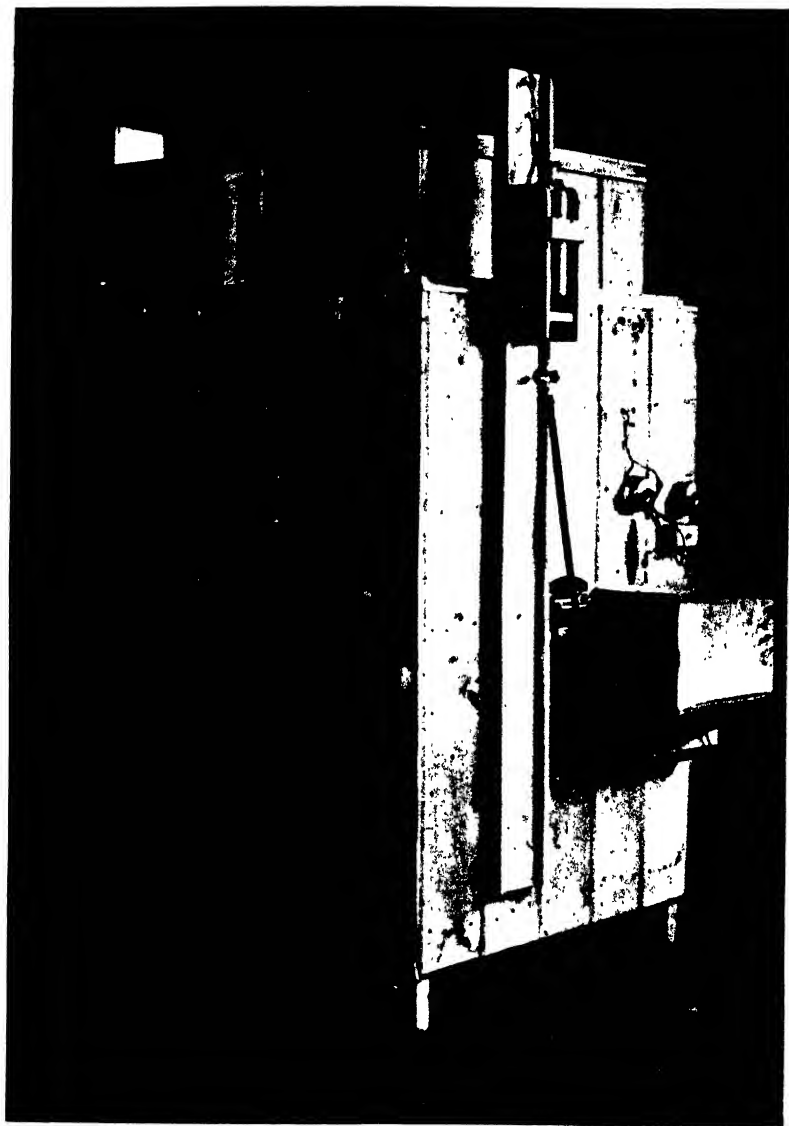
GOUGH.—FACTORS AFFECTING THE RESISTANCE OF THE FLOUR BEETLE, *TRIBOLIUM CONFUSUM* DUV., TO HYDROGEN CYANIDE (pp. 533–571)

GOUGH.— Factors affecting the Resistance of the Flour Beetle, *Tribolium confusum* Duv., to Hydrogen Cyanide (pp. 533-571)

UCHIDA, H. (1938). Relation of effectiveness of the fumigation by carbon bisulphide to the development of the egg, larva and pupa of *Chaetodacus dorsalis* Hendel. Prelim. Rep. (in Japanese), Abstract from *Rev. appl. Ent.* A, **26**, 263.

WIGGLESWORTH, V. B. (1934). *Insect Physiology.* Methuen. p. 78.

WOGLUM, R. S. (1925). Observations on insects developing immunity to insecticides. *J. econ. Ent.* **18**, 593.

EXPLANATION OF PLATES XXXI AND XXXII

Photographs of first apparatus

Plate XXXI. Apparatus closed.

Plate XXXII. Apparatus with top removed.

(Received 23 January 1939)

INVESTIGATIONS UPON THE PEA STRAIN OF *HETERODERA SCHACHTII* SCHMIDT AND ITS RÔLE IN THE CAUSATION OF PEA SICKNESS

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(With Plates XXXIII and XXXIV)

CONTENTS

1. INTRODUCTION

AMONG the nematodes of the family Anguillulinidae attacking the roots of plants of economic importance the genus *Heterodera* contains two of the most important species, namely, *H. marioni* (Cornu, 1879; Goodey, 1932), and *H. schachtii* (Schmidt, 1871). The latter species was discovered by Schacht (1859) infesting the roots of sugar beet when it was found responsible for the condition known as beet sickness (Rübenmudigkeit). Kühn (1874) reported a strain which has been discovered to attack oats, and, to a lesser degree, barley, wheat and rye. Other strains have been found to attack potatoes, hops and leguminous plants.

The last strain was first found attacking peas by Liebscher (1890), who concluded that it was a different species from the beet eelworm and named it *H. göttingiana*, now regarded as a synonym of *H. schachtii*. This pea race was later found to be associated with the fungus *Fusarium vasinfectum* Atk. var. *Pisi* van Hall (= *Fusarium oxysporum* Schl. *f.* 8

Snyder) by Capus (1917, 1918) in France. In Britain the strain was first noticed by Theobald (1912), since when it has briefly been dealt with by Edwards (1935), Triffitt (1931), Walton *et al.* (1933), as well as being reported from numerous widely distributed areas in this country (see *Reports on Pests of Crops* issued by the Ministry of Agriculture, 1928–37).

Although the strains of *Heterodera schachtii* are generally recognized to be invariably associated with the manifestation of a pathological condition of the host plants commonly known as "sickness", e.g. pea sickness, yet the majority of workers conducting investigations upon the bionomics of the nematode hold the opinion that this eelworm is only a contributory factor and that additional factors play an important part in the production of the typical syndrome of "sickness". Thus Morgan (1926), Smith & Miles (1929) and Miles (1933) concluded from their investigations upon the potato strain of *H. schachtii* that the eelworm alone is not sufficient to produce the pathological condition of the host plants known as potato sickness, while Carroll (1933) found that the intensity of this disease is directly proportional to the cyst content of the soil.

The biological races of this nematode parasitic upon sugar beet, potato and oats have been investigated by many workers both in this country and on the Continent but the pea race has been almost entirely neglected. In view of this fact and since this particular race has now become a menace to pea culture in many localities in this country, the present investigations were undertaken.

2. SYMPTOMS AND NATURE OF PEA SICKNESS

The details of the pathological changes shown by diseased plants examined during the course of the experiments were found to vary with the intensity and duration of the sickness, but throughout its progress the symptoms are characteristic. Infected plants never make good growth and are unthrifty in appearance, even if they succeed in attaining maturity and in forming pods. They are much dwarfed and their stems are weak, thin and paler in colour than those of healthy plants.

The foliage is also characteristically affected, since it is pale green or yellow in colour and presents an abnormal appearance. The weak, crumpled laminae of the leaves are never as large and strong as those of healthy plants. With the final onset of the disease, all the leaves become limp and flaccid. The upper, younger leaves remain partially unopened and are closely set together, since the apical internodes fail to elongate

because of the retardation of growth of the plant. The lower leaves are curled and withered, eventually dropping off the stem. A gradation in the pathological manifestations of the disease in the foliage can be observed from the dead, brown basal leaves to the pale green undeveloped ones at the apex of the shoot. The foliage on the whole has a distinct yellow tinge, which becomes more apparent as the disease progresses and is conspicuous when a large number of sick plants are seen together, as in a field.

The flowers are small and never as large as those on healthy plants; they are not freely exposed on account of the exceedingly short internodes of the stem and the crowding together of the leaves at the apex of the shoot. In consequence, the bloom of affected plants is situated at the apices of the shoots which, in contrast to healthy ones, branch but slightly.

The root system of a badly attacked plant is poor and impoverished, and if examined at the opportune time will be found to be heavily infected with the cystic females of *H. schachtii*. The primary root is short, and very early in the life of the plant shows a considerable degree of decay towards its base. The secondaries, besides being short, are brown and soft in texture in contrast to those of a healthy plant which are long, firm and white in colour. The bacterial nodules, so plentiful and universal upon the roots of vigorous plants, are greatly reduced in number and may be entirely absent in severe cases.

In addition to these aerial and root symptoms, the stem at ground level may be partly or completely encircled by a dark brown ring of diseased tissue which was found in the present studies to occur only in the presence of the fungus *Aphanomyces euteiches* Drechsler.

3. EXPERIMENTS ON THE DETERMINATION OF THE FACTORS INVOLVED IN THE CAUSATION OF PEA SICKNESS

A quantity of soil that was definitely known to be sick, inasmuch as pea plants grown in it the previous year were badly diseased and had shown the various pathological changes characteristic of pea sickness, was obtained. As a preliminary experiment, several samples of this soil, after it had been well mixed, were potted and planted with peas. The young plants were carefully watered and kept under constant supervision throughout the period of growth. All of them in due course became sick, wilted and died, presenting the usual facies of pea sickness.

Examination of the plants revealed that *H. schachtii* was present in

the roots in all stages of development. Another nematode, *Rhabditis brevispina* Claus (1862), was also discovered infesting the plants, but in all cases this species appeared in the tissues of dying hosts only and was never found in actively growing plants. In addition, the fungus *Aphanomyces euteiches* Drechsler (1925) was discovered invading the plant tissues at the junction of the stem and root.

Although the probable causal factors were thus ascertained, it was obviously possible that, in addition, one or more unknown factors might be involved, without which typical pea sickness would not be manifested. The problem, therefore, resolved itself into an attempt to evaluate the importance of the rôles of *Heterodera schachtii* and *Aphanomyces euteiches* and to determine the presence or absence of one or more unknown factors, and, in the former event, to assign to the factor or factors their proper place in the causation of pea sickness. It was evident that the various agencies concerned would need to be studied individually and in all possible associations with one another, and that experiments to this end would entail the isolation and combinations of the factors discovered and the potential but unknown ones.

It was assumed that all the causal factors would be removed from the infected soil by a process of sterilization; certainly the nematode cysts and the fungal spores would be non-viable after such treatment. With the object of modifying the infected soil as little as possible, sterilization by steam rather than by chemicals was chosen. In view, however, of the stimulating action of steam sterilization upon plant growth, it was evident that untreated soil would need to be utilized as a control in order to estimate the beneficial effects on the plants resulting from such treatment.

The cysts of *Heterodera schachtii* on account of their relatively large size, having an average diameter of 0·5 mm., can be removed from infested soil by sieving, but the sieved soil would still be contaminated with the fungus spores. Apart from the removal of the cysts and larger soil particles, the composition of the sick or sieved soil would be maintained, since the unknown factors, unless they were inherent in the cysts or in the coarser components of the soil, would still be present. The texture of the soil would, however, be modified by the removal of the coarser material unless it was replaced by some substitute.

The removal of the fungal spores offered insuperable difficulties, but it was possible to culture the fungus for the purpose of inoculation, so that soil previously uninfected could be infected with it as desired.

Thus the factors *Heterodera schachtii* and *Aphanomyces euteiches*, as

well as the latter plus the unknown factors, if any, could be experimented with either singly or in association with each other, but the adoption of steam sterilization made the provision of "controls" essential. For these controls, soil known to be free from both *Heterodera schachtii* and *Aphanomyces euteiches* was used. Since both the eelworm and the fungus could be inoculated into this untreated, non-infected soil, the effects of the problematical and unknown factors could be estimated by their absence.

Culture of Aphanomyces euteiches *and the preparation of inoculum*

According to Drechsler (1925), as was later confirmed in the course of the present studies, the fungus *Aphanomyces euteiches* can only be induced to grow on artificial media by its transference during the short period when it is actively growing within the tissues of the host. For this reason young seedlings showing the first symptoms of fungal attack were selected. The basal portions of the stems were removed and thoroughly washed several times in sterile water before being placed on sterile plates of prune and pea agar.

A sparse growth was obtained on a few of these plates which quickly outgrew the bacterial colonies that developed around the various pieces of stem. Subcultures were then made by transferring some of the sterile portions of the plate, traversed by hyphae, to fresh plates of maize meal agar. These were then used as stock cultures since the abundant oospores produced by the fungus, when growing in this medium, will germinate readily upon this maize meal agar and continue to grow until the dish is covered by a densely compacted mass of aerial mycelia bearing enormous numbers of oöspores. It was decided to use the maize meal cultures at the height of the production of oospores for the purpose of making inoculations, since the oospores germinate very readily in water, and healthy seedlings placed in soil mixed with a relatively large amount of inoculum readily became infected by the parasite.

Collection of the cysts of Heterodera schachtii
and their preparation for inoculation

The resistant stage of the eelworm is the durable cyst containing numerous eggs, each of which has a larva within it capable of emerging to infect a host plant, and, in order to transfer the nematode to soil formerly free from it, it was necessary to collect supplies of these cysts.

Two methods of collecting cysts had been in use by previous workers. The first, Carroll (1933), depends upon the collection of cysts from the

roots of infected plants, but this method could not be utilized in the present work. The second, Morgan (1925), is based upon the fact that the cysts will float on water. The technique consists in shaking small amounts of soil varying from 25 to 100 g. in a long-necked flask filled with water. The debris, together with the cysts, floats to the surface of the water and is then transferred to a filter paper from which the cysts are separately collected by hand. This method was improved upon in the present experiments by the use of an apparatus, which will be described in a subsequent paper, devised to extract cysts from several pounds of soil at one time.

The following method was adopted for the purpose of inoculating the various series. As the weight of soil required to fill each pot was 12 lb. this amount of pea sick soil was passed through the cyst collecting apparatus in three equal instalments. The supernatant matter, including the cysts, was sterilized by immersion in "10 volumes" hydrogen peroxide for a period of 30 min., when the surface-sterilized cysts were removed and washed in sterile water.

The cysts from each 12 lb. of affected soil were now uniformly distributed throughout the same weight of the soil to be inoculated, and the latter restored to its appropriate pot.

Triffit (1931) had employed hydrogen peroxide for the surface sterilization of *H. schachtii* cysts, but before using this technique the present writer ascertained the effects of the treatment upon the cysts and the contained larvae. Sterilized cysts were placed upon plates of nutrient agar, and since no bacterial or fungal growths developed, it was apparent that the peroxide had destroyed any extraneous spores on the surface of the cysts. Sterilized cysts were later dissected and the contained larvae found to be viable and active.

The sterilization of the surfaces of the cysts rendered the spores of *Aphanomyces euteiches* or any other organism innocuous so that only *Heterodera schachtii* would be inoculated into the soil.

Plan of pot experiments

Pot experiments seemed to offer the best method for the elucidation of the rôles played by the different factors, since each could be isolated and studied critically in relation to the plants under almost identical conditions.

Eleven series were set up. In each series, there were six 8½ in. earthenware flower pots, each holding 12 lb. of soil. All the pots had been steam sterilized and provided with adequate amounts of sterile material for drainage.

A quantity of pea-sick soil was obtained and air-dried until it could be easily pulverized. It was passed through a ½ in. mesh sieve to remove the larger stones and thoroughly mixed until its constitution was uniform as regards its general appearance and cyst concentration. When similar cyst counts on samples of identical weight from different portions of the heap of soil were obtained, using a modification of the technique described by Morgan (1925), it was considered that homogeneity with regard to cyst distribution had been attained. The soil passing through a sieve of 2 mm. mesh was taken in each case in the examination for cysts.

The different series of pots were as follows:

Series I. Untreated pea-sick soil. Apart from the preparation already described the soil used in this series received no further treatment.

Series II. Steam-sterilized pea-sick soil. Soil similar to that in series I, except that it had been steamed in a Koch sterilizer for 2 hr. at the temperature of 98° C., was used.

Series III. Sieved pea-sick soil plus sand. The soil was identical with that used in series I except that it had been passed through a sieve of $\frac{1}{60}$ in. mesh with the object of removing the eelworm cysts. In order, so far as possible, to restore its original physical composition, the soil was thoroughly mixed with clean, dry, sterilized, coarse grained sand equal in weight to the discarded residue.

Series IV. Steam-sterilized pea-sick soil inoculated with *Aphanomyces euteiches*. The soil received a treatment similar to that in series II and, in addition, a standard amount of plated agar supporting a uniformly heavy growth of *A. euteiches* was intimately incorporated with it at the time of sowing.

Series V. Steam-sterilized pea-sick soil inoculated with cysts of *Heterodera schachtii*. The soil received a treatment similar to that in series II except that it was inoculated a few days before sowing with a number of cysts equivalent to that destroyed during the process of sterilization.

Series VI. Steam-sterilized pea-sick soil inoculated with both *Aphanomyces euteiches* and *Heterodera schachtii*. The soil received a treatment similar to that in series II except that it was inoculated with equivalent amounts of *Aphanomyces euteiches* and cysts of *Heterodera schachtii*, as described in series IV and V respectively.

Series VII. Untreated non-infected soil. The soil used in this series had been proved to be free from *Aphanomyces euteiches* and cysts of *Heterodera schachtii* by previously rearing uninfected plants in samples of it. In texture and composition it was highly similar to that selected for series I and received essentially the same preparation.

Series VIII. Non-infected soil infected with *Aphanomyces euteiches*. Soil similar to that used in series VII was inoculated with an amount of *A. euteiches* equivalent to that used in series IV and VI.

Series IX. Non-infected soil inoculated with cysts of *Heterodera schachtii*. Soil similar to that in series VII was inoculated with a number of cysts equivalent to that added in series V.

Series X. Non-infected soil inoculated with *Aphanomyces euteiches* and *Heterodera schachtii*. Soil similar to that in series VII inoculated with equivalent amounts of *Aphanomyces euteiches* and *Heterodera schachtii* as used in series VIII and IX, respectively.

Series XI. Sterilized non-infected soil. Soil similar to that in series VII was steam-sterilized as described for series II.

It was known that all the conditions necessary for the production of the disease

were present in the pea-sick soil, as used in series I, since the crop sown in the field from which it was obtained had been a total failure in the previous year on account of this malady.

The sterilization of the soil was carried out in April, and on 12 May 1936, twelve healthy surface-sterilized pea seeds kept in wet, sterile sand until they had commenced to germinate, were evenly planted at a depth of 1½ in. in each pot. The plants of each series were subsequently watered whenever necessary, depending on the weather conditions, on each occasion equal volumes of water being applied to the different pots in all the series.

To ensure that ample and readily available supplies of all the necessary inorganic nutritive substances would not be lacking, an artificial fertilizer of the following composition and proportion was incorporated with the soil of each pot of every series immediately before planting:

Ammonium sulphate	19·70 g.	1 part
Potassium sulphate	39·40 g.	2 parts
Superphosphate	59·10 g.	3 parts

1·64 g. of the mixture was added to each pot of 12 lb. of soil, a dressing equivalent to 6 cwt. of fertilizer per acre of soil.

Effects of the treatments on plant growth and the manifestation of the disease

One week after planting, shoots of the seedlings had appeared above the surface of the soil in every pot. The first well marked indications of attack by *Aphanomyces euteiches*, namely the browning and weakening of the basal portions of the stem, were detected towards the end of the second week in June. The symptoms were well marked in the plants of series I, less noticeable in those of series III, and were not present in those of the artificially inoculated series, namely, Nos. IV, VI, VIII and X. The plants of these latter series were found, however, at a later date, to be infected, though never to the same degree as those in series I and III.

Towards the middle of June the effects of the various treatments were already manifesting themselves as regards the number of plants and the amount of foliage growth in the different series. The plants in series II, VII and XI showed a much more vigorous growth than those in the remaining series.

Those series which had been inoculated with *A. euteiches*, either in the absence (series IV, IX) or the presence (series VI and X) of *Heterodera schachtii*, as well as those series (I and III) which were naturally infected, included fewer plants than those not inoculated with this fungus (series V, IX).

By 18 August the plants had matured but the majority of the pods were still green. At this date the differences between the plants of the various series were more pronounced than at any time earlier in the season and brief notes on each series follow:

Series I. Untreated pea-sick soil. All the plants were definitely very sick, being stunted and with thin, weak stems varying from 1½ to 1¾ ft. in height. The leaves were small and unhealthy in colour varying from a sickly yellow near the base of the main stem to yellowish green towards its apex. The pods were small, few in number and invariably almost empty. In each pot one or more plants had died before reaching the miserable maturity attained by the remainder. In short, all the typical symptoms of pea sickness were exhibited by all the plants in this series, including the presence of the cysts of *H. schachtii* on the roots. In addition, the characteristic brown discoloration was shown at the base of the stem due to an attack by *Aphanomyces euteiches*.

Series II. Sterilized pea-sick soil. The plants were unusually vigorous and healthy with tall thick stems averaging from 3½ to 4 ft. in height, large, dark green healthy leaves and numerous well developed, full pods. Signs of disease were entirely lacking and although the plants were beginning to die down, none had perished during the season.

Series III. Sieved pea-sick soil. The plants were small and stunted, measuring from 1½ to 2 ft. high, their stems being very slender and bearing poorly developed pale green leaves. The pods were few in number and almost empty. The usual symptoms of parasitization by *A. euteiches* were present on nearly all the plants, several of which had already succumbed to the effects of this fungus.

Series IV. *A. euteiches* inoculated into sterilized pea-sick soil. The plants were healthier in appearance than those in series I and III. They measured 2¼ ft. or more in height and possessed pale green foliage.

Series V. *Heterodera schachtii* inoculated into sterilized pea-sick soil. The plants were obviously diseased, the foliage being very pale and the yield of crop exceedingly small. The heights of the plants averaged about 2 ft.

Series VI. *Aphanomyces euteiches* and *Heterodera schachtii* inoculated into sterilized pea-sick soil. The plants were even poorer in appearance than those in series IV and V, indicating that the combined effects of both parasites were greater than those due to either alone.

Series VII. Untreated non-sick soil. The plants evidenced no pathological symptoms of any kind and measured on an average 3–3½ ft. in height. The stems were well developed and the leaves normal in colour. Some individual plants were still flowering though the greater number of them carried well filled pods. The plants, though exhibiting a good healthy growth, were inferior in appearance to those of series II and XI.

Series VIII. Non-sick soil inoculated with *Aphanomyces euteiches*. The plants were on the whole fewer in number and not so flourishing as those in series VII. The foliage was of a dull green colour and the pod production rather poor. Symptoms of fungal attack were not, however, very evident.

Series IX. *Heterodera schachtii* inoculated into non-sick soil. The plants, though somewhat variable in appearance in the different pots, were definitely retarded in

comparison with those of series VII. They measured only about 2½ ft. in height and showed all the typical symptoms of pea sickness with the exception of the dark discoloration at the base of the stem, which is associated with *Aphanomyces euteiches*.

Series X. *Aphanomyces euteiches* and *Heterodera schachtii* inoculated into non-sick soil. This series included plants which were inferior to those of series VII, VIII and IX. The whole symptoms of disease were not, however, generally so well marked as in series VI.

Series XI. Sterilized non-sick soil. The plants were definitely superior to those in series VII and comparable in all respects with series II. The general appearance, as in series II, gave the impression of great robustness and unusual vigour both of the vegetative portions and of the pods, thus demonstrating the beneficial effects of the process of steam sterilization of the soil.

Pls. XXXIII and XXXIV, which were taken in July, illustrate the effects of the various treatments upon the plants of the different series. It will be noted (fig. 1) that the symptoms of pea sickness were very marked in the plants in the pot from series I, particularly the dwarfed, thin stems and the poor pale coloured foliage, and the luxuriant growth and fine development of the plants of series II is in marked contrast. It is evident, therefore, that the pathogenic factors were removed by the process of steam sterilization and that the plants were more than usually robust. The pot from series III shows diseased plants which were superior to those of series I, but much inferior to those in series II. It may be mentioned in this connexion that the replacement of the coarse gravel removed from the soil by sieving was not adequately compensated for by the addition of an equal weight of sand. In contrast to the soil in the other ten series, the mixture of sand and sieved soil in this series resulted in the formation of a tenacious, close grained compost which was almost impermeable to water, thus depriving the plants growing in it of an abundant supply of moisture and air for their normal development. The plants of this series, as already stated, were badly infected by *Aphanomyces euteiches* which was probably aided in its parasitization by the tenacious character of the soil.

The plants of series IV, V and VI, which are shown in fig. 2, illustrate the adverse effects of the fungus and the eelworm, acting separately or in conjunction with one another. It is apparent that the combined effects of both parasites were cumulative and that the plants of all three series were considerably inferior to those of series II (fig. 1).

Similarly, fig. 3, showing a specimen pot of each of series VIII, IX, and X also illustrates a sequence of the cumulative effects of these two parasites. It is evident that the plants in all these pots are considerably less vigorous than those of the untreated series (VII), which is shown in

fig. 4; the adverse influences of the fungus and the nematode were again very marked and of the two organisms, the latter proved to be the more important pathogenic agent. The sickness is more pronounced in the inoculated unsterilized series (VIII, IX and X), than in the corresponding inoculated sterilized series (IV, V and VI). This difference is attributable to the stimulative effect which the process of sterilization has upon plant growth.

Fig. 4 shows typical pots from series IX, VII and XI and demonstrates the effect of *Heterodera schachtii* alone. In contrast to the plants of series VII and XI, those of series IX are obviously sick. There can be no doubt, therefore, of the significance of the nematode as the causative agent of pea sickness, while the effect of steam sterilization in stimulating plant growth to a marked degree is well shown.

4. CONCLUSIONS

The results of the experiments described show that in the pea-sick soil studied, the eelworm *Heterodera schachtii* and the fungus *Aphanomyces euteiches* were both concerned in the production of a diseased condition of pea plants in the absence of any other factor of an organic nature. Either of these organisms can produce pathological symptoms in the absence of the other, and the effect upon the plants of infection by *Heterodera schachtii* alone indicates that it is the primary factor causing pea sickness.

5. SUMMARY

1. A series of experiments was carried out upon the etiology of pea sickness, a disease which is invariably associated with *Heterodera schachtii*.

2. In addition to this eelworm, a fungus, *Aphanomyces euteiches*, was found to be concerned in the production of the diseased condition of the plants in the soil studied.

3. Both *Heterodera schachtii* and *Aphanomyces euteiches* can singly produce a pathological condition of the host in the absence of any other pathogenic factor.

4. The effects upon the plants of attack by *Heterodera schachtii* alone indicate that it is the primary cause of pea sickness.

I am greatly indebted to Mr E. E. Edwards, M.Sc., Department of Agricultural Zoology, University College, Cardiff, wherein these researches

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determination of the identity of *Aphanomyces euteiches*.

REFERENCES

Capus, J. (1917). Sur le dépérissement des cultures de pois en Gironde de la double action de l'*Heterodera schachtii* et du *Fusarium vasinfectum*. *Bull. Soc. Zool. Agric. Bordeaux*, **16**, 70, 87.
—— (1918). Invasion des cultures de pois en Gironde par *Heterodera schachtii* Schmidt. *Ann. Epiphyt.* **5**, 239.
Carroll, J. (1933). A study of the potato worm, *Heterodera schachtii* in the Irish Free State. *J. Helminth.* **11**, 137.
Claus, C. (1862). Ueber einige in Humus lebende Anguillulinen. *Z. wiss. Zool.* **12**, 354.
Cornu, K. (1879). Études sur le *Phylloxera vastratrix*. *Mém. prés. Acad. Sci., Paris*, **26**, 1–357.
Drechsler (1925). Root rot of peas in the United States caused by *Aphanomyces euteiches* (n.sp.). *J. agric. Res.* **30**, 293.
Edwards, E. E. (1935). On *Heterodera schachtii*, with special reference to the oat race in Britain. *J. Helminth.* **13**, 129.
Goodey, T. (1932). On the nomenclature of the root-gall nematodes. *J. Helminth.* **10**, 21.
Kühn, J. (1874). Ueber das Vorkommen von Rübennematoden an den Wurzeln der Halmfrüchte. *Z. Ver. Rübenzucker Ind. Zollver.* **24**.
Liebscher, C. (1890). Eine Nematode als Ursache der Erbenmüdigkeit des Bodens. *Dtsch. landw. Pr.* **17**, 436, 672.
Miles, H. W. (1933). Field studies on *H. schachtii* Schmidt, in relation to the pathological condition known as "Potato sickness". *J. Helminth.* **8**, 103.
Morgan, D. O. (1925). Investigations on eelworm in potatoes in south Lincolnshire. *J. Helminth.* **3**, 185.
—— (1926). Some remarks on the etiology of potato sickness in Lincs. *J. Helminth.* **4**, 49.
Schacht, H. (1859). Ueber einige Feinde und Krankheiten der Zuckerube. *Z. Ver. Rübenzucker Ind. Zollver.* **9**, 390.
Schmidt, A. (1871). Ueber den Rüben-Nematoden (*Heterodera schachtii* A.S.). *Z. Ver. Rübenzucker Ind. Zollver.* (V. Sch), **21**, 1.
Smith, A. M. & Miles, H. W. (1929). Investigations on *Heterodera schachtii* in Lancashire and Cheshire. Pt. III. *Ann. appl. Biol.* **16**, 596.
Theobald, F. V. (1912). Report on economic zoology for year ending 30 September 1912. *J. S.-E. Agric. Coll.* no. 21, p. 173.
Triffitt, M. J. (1931a). On the pathogenicity of *Heterodera schachtii* to potatoes and mangolds. *J. Helminth.* **9**, 1.
—— (1931b). On the eelworm *Heterodera schachtii* attacking peas in Britain. *J. Helminth.* **9**, 175.
Walton, C. L., Ogilvie, L. & Mulligan, B. O. (1933). Observations on the pea strain of the eelworm *Heterodera schachtii* and its relation to "pea-sickness". *Rep. agric. hort. Res. Sta. Bristol*, p. 74.

EXPLANATION OF PLATES XXXIII AND XXXIV

PLATE XXXIII

Fig. 1. Series I. Pea plants grown in "pea-sick" soil. Series II. Pea plants grown in sterilized "pea-sick" soil. Series III. Pea plants grown in sieved "pea-sick" soil.

Fig. 2. Series IV. Pea plants grown in "pea-sick" soil, sterilized and reinfected with *Aphanomyces euteiches*. Series V. Pea plants grown in "pea-sick" soil, sterilized and reinfected with *Heterodera schachtii*. Series VI. Pea plants grown in "pea-sick" soil, sterilized and reinfected with both *Aphanomyces euteiches* and *Heterodera schachtii*.

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PLATE XXXIV

Fig. 3. Series VIII. Pea plants grown in eelworm and fungus-free soil infected with *Aphanomyces euteiches*. Series IX. Pea plants grown in eelworm and fungus-free soil infected with *Heterodera schachtii*. Series X. Pea plants grown in eelworm and fungus-free soil infected with both *Aphanomyces euteiches* and *Heterodera schachtii*.

Fig. 4. Series IX. Pea plants grown in eelworm and fungus-free soil infected with *Heterodera schachtii*. Series VII. Pea plants grown in eelworm and fungus-free soil. Series XI. Pea plants grown in sterilized eelworm and fungus-free soil.
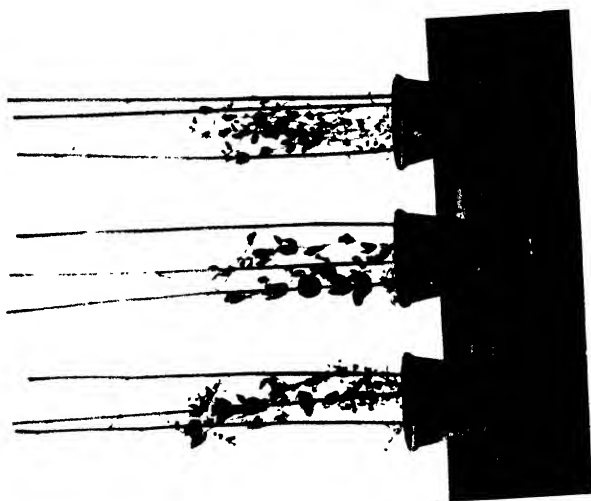
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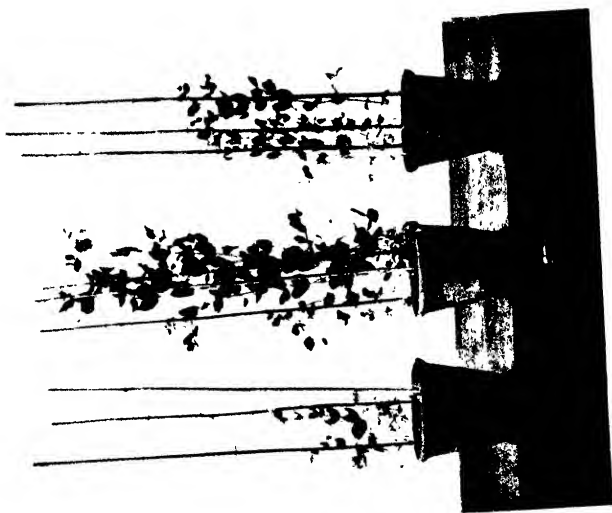
Fig. 1.

POWELL. —INVESTIGATIONS UPON THE PEA STRAIN OF *HETERODERA SCHACHTII* SCHMIDT AND ITS RÔLE IN THE CAUSATION OF PEA SICKNESS (pp. 572–584)
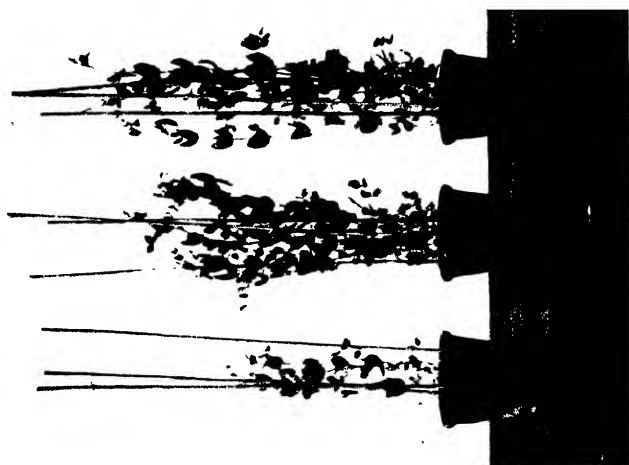
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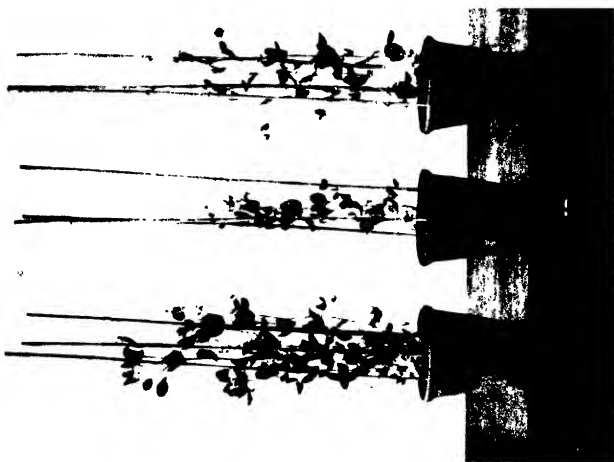
Fig. 3.

POWELL.—INVESTIGATIONS UPON THE PEA STRAIN OF *HETERODERA SCHACHTII* SCHMIDT AND ITS RÔLE IN THE CAUSATION OF PEA SICKNESS (pp. 572–584)

THE TOXICITY OF POISONS APPLIED JOINTLY[1]

By C. I. BLISS

Mexico, D.F.

(With 14 Text-figures)

CONTENTS

INTRODUCTION

WITH the development of quantitative methods for the estimation of toxicity, a study of the mode of action of poisons applied jointly has acquired new potentialities. Some of this interest is theoretical and concerns the statistical approach to the relation between dosage and percentage kill. The typical curve relating these two variables is asymmetrical and sigmoid, but it can be transformed to a straight line and computed with measurable precision by methods first introduced by Gaddum (1933). Empirical tests have shown the wide applicability of the technique to a large number of animal species and poisons. Cases have been reported, however, in which the slope of the rectified dosage-mortality curve has changed more or less abruptly, usually at the lower dosages. When the population of test animals was reasonably uniform, the most general explanation of these "breaks" has been that each segment of the rectified curve represented a specific type of toxic action, such as might be produced by one constituent in a complex poison. It is desirable to show, therefore, what would result with different mixtures that are assumed or known to contain two toxic ingredients.

The subject is also of practical interest. In the search for new insecticides combined poisons offer many possibilities, but criteria are

needed for separating mixtures in which the combined ingredients possess an enhanced toxicity from others in which they act independently since the former group provides the more promising field of investigation. Those investigating these possibilities have not yet agreed either upon experimental procedure or upon the meaning of their results. In a study on the toxicity of rotenone-pyrethrin sprays, for example, Le Pelley & Sullivan (1936) concluded that synergistic action was absent. Yet, in a footnote based upon the same original data, H. H. Richardson found definite evidence of synergism. To resolve uncertainties of this nature the quantitative problems underlying the toxicity of combined poisons and drugs should be examined in more general terms than heretofore. With this in view the present paper is concerned primarily with the definition and quantitative analysis of three types of joint toxic action in which the percentage mortality is used as the measure of response.

Types of joint action

The three types of joint action can be identified from the relative toxicities of the separate constituents when applied singly and in mixtures. The most general measure of toxicity and the only one considered here is the percentage kill and the dosage-mortality curve based upon it. The computation of this curve has recently been summarized by Bliss (1938). As the simplest case we will assume that the characteristic curve for each constituent in a given mixture can be plotted as a single straight line when the dosage is transformed to logarithms and the observed percentage mortality to probits or their equivalent. The equation of the curve can then be written in the simple form

$$Y = a' + b \log D, \qquad \qquad \ldots\ldots(1)$$

where Y is the expected mortality in probits at any given dosage D, and a' and b are constants computed from the experimental data. The regression coefficient b measures the rate of change in mortality per unit change in the log dose, and its reciprocal, $1/b$, is the standard deviation of sensitivity (or resistance) to the drug or poison. The degree of toxicity, which is determined by a', is independent of the slope or standard deviation, but it is usually more convenient to measure toxicity in terms of the median lethal dose (LD 50) or its logarithm rather than in terms of a'. To obtain log LD 50 from equation (1), Y is set equal to 5 and the equation solved for log D. As the mid-point of the complete curve it is the standard measure of toxicity.

When two poisons are combined or administered jointly, we may

specify further that the toxicity of the mixture must be determined at several dosages. This is now standard procedure in many cases. It has a twofold objective: (1) to determine whether the dosage-mortality curve for the mixture is linear over the full range of mortalities, a point of diagnostic value, and (2) to compute the amount of mixture required to kill fifty or some other specified percentage of the test animals. Usually the ratio of the constituents can be kept constant by preparing a standard solution which is then diluted to obtain the required series of doses. It is essential, of course, that the components be compatible chemically under the conditions of the experiment. If they react to form a stable compound prior to application, this is a complication beyond the present scheme, for study by a chemical technique. The classification below is based entirely upon relative toxicities.

With these limitations, we may recognize three principal types of joint action:

(1) *Independent joint action.* The poisons or drugs act independently and have different modes of toxic action. The susceptibility to one component may or may not be correlated with the susceptibility to the other. The toxicity of the mixture can be predicted from the dosage-mortality curve for each constituent applied alone and the correlation in susceptibility to the two poisons; the observed toxicity can be computed on this basis whatever the relative proportions of the components.

(2) *Similar joint action.* The poisons or drugs produce similar but independent effects, so that one component can be substituted at a constant proportion for the other; variations in individual susceptibility to the two components are completely correlated or parallel. The toxicity of a mixture is predictable directly from that of the constituents if their relative proportions are known.

(3) *Synergistic action.* The effectiveness of the mixture cannot be assessed from that of the individual ingredients but depends upon a knowledge of their combined toxicity when used in different proportions. One component synergizes or antagonizes the other.

These three types of joint action are sufficiently well defined that they can be distinguished with little difficulty, although there probably are other cases which fall between or outside of these classifications.

Independent joint action

The simplest case is that in which the two components act independently of each other. Presumably each involves a characteristic and distinct chain of reactions in the organism, so that the animal dies from

the failure of a different vital system in the one case than in the other. Susceptibility to the one ingredient, however, may still be correlated to a greater or lesser degree with susceptibility to the other. Hough (1934) has shown, for example, that a race of codling moth which was more susceptible to ingested lead arsenate was also more susceptible to poisoning by hydrocyanic acid gas. Any estimate of the mortality expected from two poisons which act independently must take this factor into account.

The combined effect of two poisons, A and B, which act independently can be examined most readily in terms of the proportion killed. If there were no correlation in susceptibility to the two components, so that the coefficient of association, r, is equal to zero, then

$$p_C = p_A + p_B - p_A p_B, \qquad \ldots\ldots(2)$$

where p_C is the proportion of animals killed by the combination when p_A and p_B are the proportions dying from the same amounts of A and B applied alone. Entomologists will recognize this as equivalent to the so-called "Abbott's formula" for computing from the observed total mortality the effectiveness of a poison, p_B, in the presence of a given natural mortality, p_A. For this purpose it is usually written as

$$p_B = \frac{p_C - p_A}{1 - p_A}. \qquad \ldots\ldots(2a)$$

If, on the other hand, there should be an exact parallelism in the susceptibility to the two poisons, so that $r = 1$, then any individual receiving a fatal dose of the less toxic ingredient would receive a more than fatal dose of the more toxic ingredient,

$$p_C = p_A, \qquad \ldots\ldots(3)$$

where $p_A > p_B$, and the combined mortality would be the same as that attributable to the more toxic ingredient applied alone. Any more general equation which will cover intermediate degrees of association as well must reduce to equation (2) when $r = 0$ and to equation (3) when $r = 1$. These requirements are met most simply by

$$p_C = p_A + p_B (1 - p_A) (1 - r), \qquad \ldots\ldots(4)$$

where $p_A > p_B$. Any case in which the mortality due to a combination poison can be estimated by equation (4) from that of the two ingredients applied separately, regardless of the percentage composition or dosage of the mixture, can be classified in this category of independent joint action.

The association between susceptibilities, r, can be computed readily from experimental data. Three dosage-mortality curves are required, one

for each of the two poisons applied alone and a third for the two applied jointly. A convenient mixture for this purpose is one in which A bears the same ratio to B as the LD50 of A to the LD50 of B, although mixtures containing other fixed proportions of the two ingredients can be used as well. The LD50 is computed from the dosage-mortality curve for each ingredient applied alone and the logarithm of their sum inserted in the equation of the curve for the combination to compute the expected kill in probits. This is then transformed to the proportion dead, p_C, by reference to a table of probits and the association of susceptibilities, r, computed from equation (4) as $r = 3 - 4\,p_C$. Within the limits of experimental error it should be possible to compute the mortality observed with any combination from the dosage-mortality curves for the separate ingredients and a single coefficient of association. Combinations which fail to meet this requirement belong to another category of joint action.

Dosage-mortality curves for hypothetical mixtures. It is assumed in part of the above procedure that the percentage mortalities, obtained with different dosages of a toxic mixture, can be plotted and computed in terms of straight lines after transformation of the observed percentages to probits and of the combined dosages to logarithms. Since departures from linearity might prove of diagnostic value, the limitations to this assumption are of interest. It can be tested conveniently by plotting the expected mortality in probits against the log dose for mixtures of two hypothetical poisons. For this purpose the association in the susceptibility to the two poisons has been assumed to be 0·6, a value intermediate between complete association and its entire absence. Combinations of poisons involve several distinct possibilities.

The first point concerns the effect of different proportions of two ingredients upon the toxicity of the mixture. In order to emphasize the contrast between them, the LD50 of the two poisons has been selected at 10 and at 2 mg. for A and B respectively, with a fourfold difference in slope. The dosage-mortality curves for the ingredients were $Y_A = 2 \cdot 5 + 2 \cdot 5 X_A$ and $Y_B = 1 \cdot 99 + 10 X_B$, where Y is the mortality in probits and X the dose in logarithms. Four mixtures have been postulated, containing the following proportions of the two ingredients in terms of their LD50's: (1) $\frac{1}{5}$ of A to $\frac{4}{5}$ of B, (2) $\frac{2}{5}$ of A to $\frac{3}{5}$ of B, (3) $\frac{3}{5}$ of A to $\frac{2}{5}$ of B and (4) $\frac{4}{5}$ of A to $\frac{1}{5}$ of B. The mortality attributed to each component if given alone was then computed for various dosages of the mixtures from the above equations and transformed from probits to p_A and p_B, which were substituted in turn in equation (4) with $r = 0 \cdot 6$ to find p_C, the combined mortality when applied jointly. After conversion

to probits the curves for the four mixtures have been plotted in Fig. 1 over a wider range of toxicities than would be determined in most experiments.

The most striking characteristic of Fig. 1 is that the dosage-mortality relation was not a smooth curve but fell into two segments which would be indistinguishable from straight lines if determined from actual biological data. Except for the mixture containing the largest proportion of the less active ingredient, the transition or "break" from one segment to the other was relatively abrupt. In other examples, not given
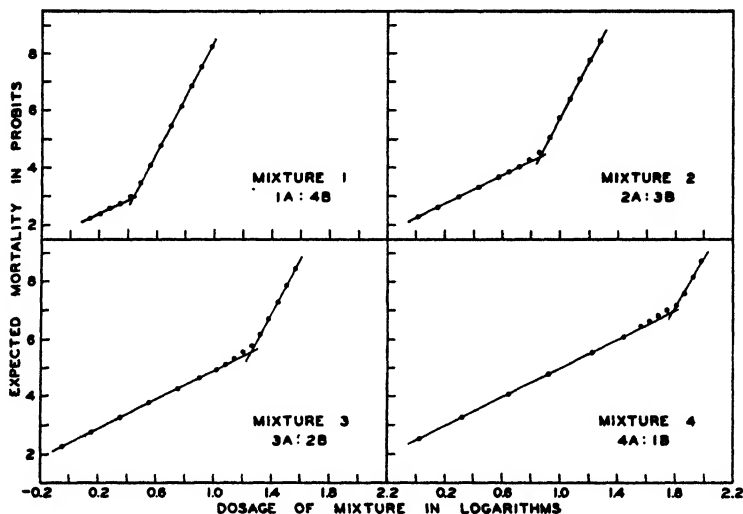
Fig. 1. The toxicity of four hypothetical mixtures containing specified proportions in equitoxic units of ingredients A and B on the assumption of independent joint action.

here, this break became more abrupt with larger values of the association coefficient, r, until with complete association between susceptibilities the slope changed at a sharp angle with no trace of curvature. Complete absence of correlation, on the other hand, increased the curvature at the break very little over that shown in Fig. 1. When the components differ in slope and act independently, therefore, the dosage-mortality curve obtained with a combined poison should contain clear evidence of its compound nature.

The extent to which the slope of each segment paralleled that of the constituent with which it is identified has been tested by computing (without weights) the straight lines drawn through each apparently linear series of plotted points. The flatter section in each case was

virtually uninfluenced by the other component, so that the original slope of $b = 2 \cdot 5$ recurred unchanged. Similarly, the slope of the steeper segment departed very little from the value for constituent B ($b = 10$) unless the break occurred at a relatively high mortality, the regression coefficients for mixtures 1–4 being 9·97, 9·97, 9·56 and 8·75 respectively. When the break occurred at less than 5 probits, the slope of the upper segment also isolated a single phase of toxic action from the complex.

The mixtures tested in Fig. 1 were based upon a single pair of hypothetical poisons. In order to test these conclusions with mixtures in-
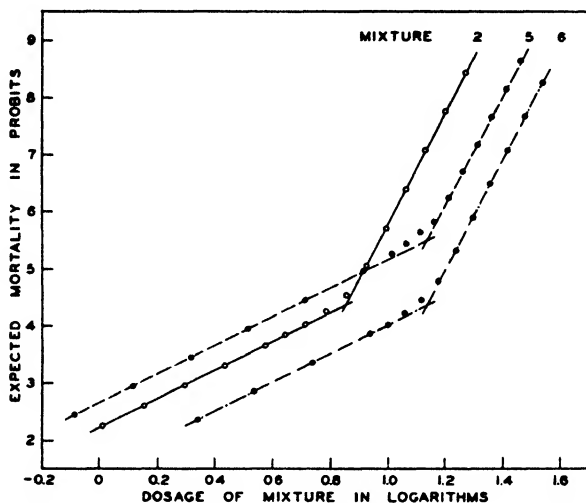
Fig. 2. The toxicity on the assumption of independent joint action of three hypothetical mixtures in which the LD50 of the ingredient with the steeper slope ($b = 10$) relative to that with the lesser slope ($b = 2 \cdot 5$) was taken to be greater (mixture 2), smaller (mixture 5) and the same (mixture 6). By definition there were 2 equitoxic units of the less toxic ingredient to 3 of the other.

volving other relations between the ingredients, three other combinations have been computed and plotted in Figs. 2 and 3. In each instance the mixture was assumed to contain 2 parts of A to 3 parts of B in terms of the LD50, quite as in the second mixture of Fig. 1 (repeated in Fig. 2 for comparison). The LD50's of the two poisons in mixture 5 were assumed to be the same as in mixture 2, but their slopes were interchanged so that the less toxic ingredient had the steeper slope, the dosage-mortality curves then being $Y_A = -5 + 10X_A$ and $Y_B = 4 \cdot 2475 + 2 \cdot 5X_B$. Although both mixtures 2 and 5 had nearly the same median lethal dose and the same general form, mixture 5 was more toxic below LD50 and mixture

2 above. In mixture 6 both ingredients were assumed to have the same LD 50 (10 mg.), but the slope of one ingredient was taken to be four times that of the other. There was the same break in the dosage-mortality curve for the combination as when the components differed in toxicity and the LD 50 for the mixture was larger than that for either alone. Finally, in mixture 7 (Fig. 3, Type I) both components were assumed to have the same slope ($b = 5$) but to differ fivefold in toxicity. In this case the break observed with all other mixtures disappeared and the slope of the combination ($b_C = 5 \cdot 150$) approached that of the original ingredients.
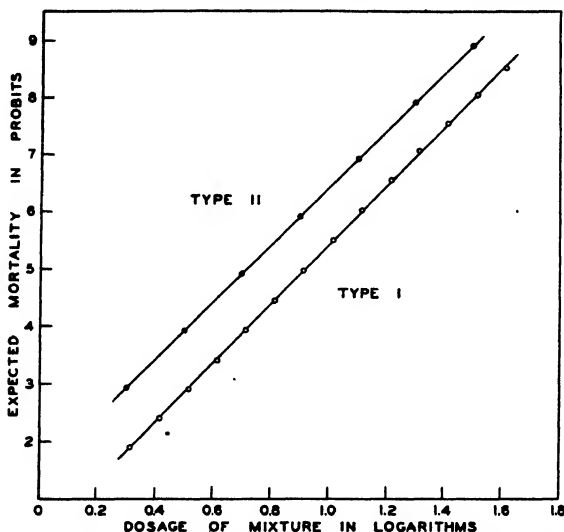
Fig. 3. The toxicity of a hypothetical mixture containing 2 equitoxic units of A to 3 of B where the LD 50 of B was one-fifth that of A but both had the same slope. They were assumed to act independently as postulated for Type I (equation (4)) in the lower curve and for Type II (equation (5)) in the upper curve.

It is evident from the above models that the dosage-mortality curve for any mixture in which the ingredients act independently will be discontinuous unless the curves for the individual components are parallel. It is also clear that recognizable characteristics of the original ingredients can be recovered quantitatively from the toxicological relations of the complex.

Experimental examples and discussion. Dosage-mortality curves resembling the models in Figs. 1 and 2 occur not infrequently in toxicological research. A convenient example has been given in a recent paper by Murray (1938) on the toxicity of a pyrethrum-kerosene spray to

female house-flies. The curve in Fig. 4 has been computed from his totals, which averaged 440 flies per concentration, and the section for the lower five concentrations has been determined separately from the upper six by the standard procedure. Pyrethrum extract is a mixture containing two principal ingredients which occur naturally together, known as pyrethrin I and pyrethrin II, pyrethrin I being much more toxic to house-flies than pyrethrin II. If we assume that Fig. 4 is a true picture of the dosage-mortality relations for a homogeneous population of female flies with this sample of pyrethrum, for which the relative proportions of pyrethrin I and II were not given, possibly the simplest explanation
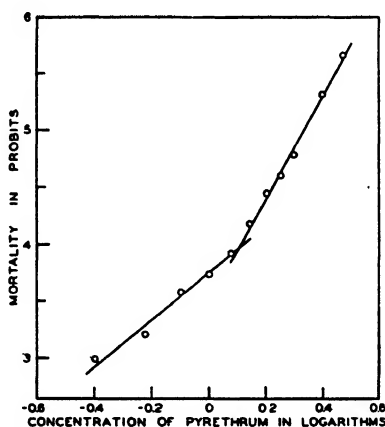
Fig. 4. The toxicity in a Peet-Grady chamber of atomized pyrethrum spray to female house-flies. Data of Murray (1938).

of the change in slope is that the lower flatter segment, for which $Y = 3.628 + 2.065(X + 0.0575)$, has been determined principally by pyrethrin II and the steeper segment, for which $Y = 4.836 + 4.530$ $(X - 0.2981)$, by pyrethrin I. On this hypothesis the two components acted independently, but from the relative abruptness of the change in slope individuals that were more susceptible to one ingredient were probably also more susceptible to the other in more or less the same order. Since this pyrethrum mixture was 3.24 ± 0.03 times as toxic to males as to females at LD50, the smallest concentration killed 23 % of the male flies, which was well above the level of mortality at which the change in slope occurred for the more resistant females. It was to be expected, therefore, that the male curve (not given here) would have a single slope over the present range of dosages, as was observed. The

hypothesis that the two segments of the female curve represent the two forms of pyrethrin is, of course, only one of several possibilities. Its correctness could be tested by determining under strictly comparable conditions the dosage-mortality curve for female flies with mixtures containing different proportions of pyrethrin I and II and especially with pure solutions of each ingredient if the latter are obtainable. Then the larger the proportion of pyrethrin II, the higher should be the probit mortality at which the change in slope occurs.

Of more general significance is the resemblance of the models shown in Figs. 1 and 2 to occasional discontinuous curves obtained experimentally with a single toxic agent. Bliss (1935) has suggested that if the amount of poison fixed by the organism were limited by adsorption, a change in the slope of the dosage-mortality curve might be explained in terms of the Langmuir adsorption theorem. Breaks of this sort occur, however, with many toxic agents where adsorption is not a factor, such as with lethal temperatures and X-rays. A more inclusive explanation of discontinuities in the dosage-mortality curve follows directly from Figs. 1 and 2.

Let us assume that a given toxic agent acts destructively and more or less independently upon two or more physiological systems in the animal. If these effects are quite distinct, it is not unlikely that they will differ also in their standard deviations (the reciprocal of slope). Then the dosage-mortality curve for a single poison could show a change in slope quite equivalent to that obtained by a mixture of two independent poisons, each with a single dominant effect. This explanation, of course, is that commonly known as the principle of the master reaction with the proviso that the limiting reactions exposed by the dosage-mortality curve must be distinct processes and not interdependent steps in a single continuous series. If these separate phases were merely links in a single chain, one would not expect the result to be independent of the relative proportions of the two ingredients as is postulated in the model.

This interpretation finds a useful extension to time-mortality relations such as are encountered in cage tests with insecticides, where insects are confined on poisoned food and the length of survival timed for each individual. Under such conditions it is common experience for the logarithm of the survival time to follow the normal distribution, so that it can be plotted as a straight line when the accumulated percentage mortality is transformed to probits and the elapsed time to logarithms, as has been described elsewhere (Bliss, 1937). The individuals in the control cages on standard food, however, usually die off at quite a different rate, so that in terms of probits and logarithms the curve is smoothly concave. A typical

series of each type has been taken from the cage tests by Boyce (1931) on the walnut-husk fly and plotted in Fig. 5. The straight line (A) shows the course of mortality on a sweetened fluosilicate dust and the curve (B) that on powdered sugar. The flies on the poisoned dust died from a single predominant toxic effect, those in the control cage from the multiplicity of causes involved in "natural mortality". Since these latter are probably diverse and presumably independent of each other, a curve such as Fig. 5 B is precisely what one would expect if the two toxic agents acting jointly in Figs. 1 and 2 were increased to five or ten or more which differ in their slope or standard deviation.
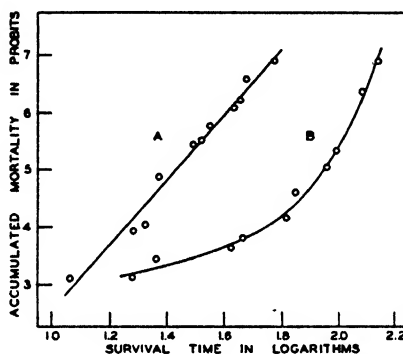
Fig. 5. Time-mortality curves for thirty-six walnut-husk flies confined with a sweetened dust containing 40 % of barium fluosilicate (A) and for thirty-five flies in a similar cage with powdered sugar (B). Data of Boyce (1931).

Similar joint action

The second type of joint toxic action is that in which two poisons produce similar but independent effects, so that in a mixture one can be substituted at a constant ratio for any proportion of the other without altering the toxicity of the combination. Since both ingredients act upon the same system of receptors within the animal, the dosage-mortality curves for both components and for any mixtures of the two should be parallel although they may differ materially as to median lethal dose. The curve for the mixture should show only such discontinuities or breaks as occur when the ingredients are tested alone. It follows from the criterion of independence that the toxicity of any combination, regardless of its percentage composition, can be predicted from the dosage-mortality curves of the ingredients. Presumably, the sensitivity to one poison is completely correlated with the sensitivity to the other, so that no

coefficient of association need be determined. Toxic mixtures of this type have a greater expected potency than those in the preceding class.

The quantitative relations of the combined poisons follow relatively simple forms. The dosage-mortality curve for a mixture of A and B is the same as that for A alone when computed in the form

$$Y_C = a' + b \log (D_A + kD_B), \qquad \ldots\ldots(5)$$

where D_A and D_B are the doses of A and B in the mixture and k is the ratio of toxicities of the separate ingredients. The ratio k is equal numerically to the anti-logarithm of a more general term, the log ratio of potencies, M. It is computed from the means of the dosage-mortality curves for the two ingredients as

$$M = \bar{x}_A - \bar{x}_B + \frac{\bar{y}_B - \bar{y}_A}{b_C}, \qquad \ldots\ldots(6)$$

where $\bar{x}_A$ and $\bar{x}_B$ are the mean log dosages and $\bar{y}_A$ and $\bar{y}_B$ the mean probits for the components A and B and b_C is the combined slope of the two curves. This equation is equivalent to equation (29) in Bliss (1935), to which the reader is referred for the standard error of M, s_M.

The dosage D_C, of a given mixture which will have a specified toxicity, can be estimated from the dose of ingredient A producing the same mortality, D_A, by a simple transformation of equation (5) to

$$D_C = \frac{D_A}{Q + k(1-Q)}, \qquad \ldots\ldots(7)$$

where Q is the proportion of A in the mixture. If the poisons can be applied only in combinations of known percentage composition which can be varied, k can be computed from the ratio of potencies for two different mixtures by means of equation (7). The value so obtained can then be applied to data from still other mixtures to test its consistency with the second type of joint action.

The greater toxicity of a combination drug or poison belonging to this as compared with the preceding class is illustrated by the two dosage-mortality curves in Fig. 3. The dosage-mortality equations for the separate components were assumed to be $Y_A = 0 + 5X_A$ and $Y_B = 3\cdot495 + 5X_B$ with $k = 5$. Two dosage-mortality curves have been computed for a mixture containing 2 parts of A to 3 parts of B in terms of the LD 50 or $Q = 0\cdot7692$. When the two ingredients were assumed to kill in diverse ways as determined by equation (4) with $r = 0\cdot6$, $Y_C = 0\cdot258 + 5\cdot150X_C$; under similar joint action as determined by equation (7), $Y_C = 1\cdot42 + 5X_C$. Some 38 % less of the combined poison would be needed to produce

equal mortalities in this case if the mixture followed the second rather than the first type of joint action.

Numerical example. As a numerical example we may examine the toxicity to adult house-flies of alcoholic solutions of rotenone and a single sample of pyrethrum, applied alone and in combination as reported by Le Pelley & Sullivan (1936). The mean probit mortality for 8–10 determinations with 100 flies each has been adopted as the measure of response to each of the five concentrations in every dosage-mortality curve. When plotted against the log concentration of the active principles in a given quantity of solution, the dosage-mortality curves for rotenone, pyrethrin and combinations of the two were parallel straight lines within the experimental error. Although the two poisons are known to have qualitatively different effects, the parallelism of their dosage-mortality curves and the conclusion reached by the experimenters suggested that mixtures of the two might agree with the second type of joint action. Since all of the combined mortalities exceeded 4 probits and thus fell within the range where, from a preceding example, the effect of pyrethrin I would be expected to dominate that of pyrethrin II, the rotenone-pyrethrin mixtures could be tested as if they were two-poison systems of pyrethrin I and rotenone.

The experiment consisted of two series of tests. Since the flies in the second series showed a somewhat greater susceptibility than those in the first, direct comparisons were limited to curves in the same series. Both ingredients were tested alone in each series. Since their relative toxicities agreed, the two estimates of M have been combined to give $M = 0.780 \pm 0.018$ or $k = 6.03$. By multiplying all concentrations of rotenone in mg. per c.c. by 6.03, the data on rotenone could be superimposed over that for pyrethrum in each series as shown in Fig. 6. A dosage-mortality curve has been computed for each series on a pyrethrum level, that for series I being $Y = 5.193 + 3.844 \ (X - 1.016)$ and that for series II, $Y = 5.249 + 3.498 \ (X - 1.011)$. If the joint potency of rotenone-pyrethrin solutions conformed to the second type, they should agree with these equations and the solid lines in Fig. 6 when converted to a pyrethrum basis.

Two different proportions of rotenone and pyrethrum were tested, a mixture in which the amounts of each component were of approximately equal potency, represented by the five concentrations in series I, and a second mixture containing a little less than 4 equi-toxic parts of pyrethrin to 1 of rotenone, represented by the five concentrations in series II. The observed kill in probits at each concentration in the two series has been plotted against $\log (D_A + 6.03 D_B)$, where D_A was the concentration of

pyrethrin and D_B of rotenone in mg. per c.c., as shown by the black circles and broken lines in Fig. 6. The mixture in series II had essentially the same toxicity as the components applied alone, but that in series I was consistently more toxic than the two constituents. The significance of the difference has been tested by computing from the dosage-mortality curves for the mixture and its components the log ratio of potencies, M, and its standard error, s_M. For series I, $M = -0.0496 \pm 0.0208$ with $t = 2.380$ and $n = 11$, a value which could occur by chance less than once
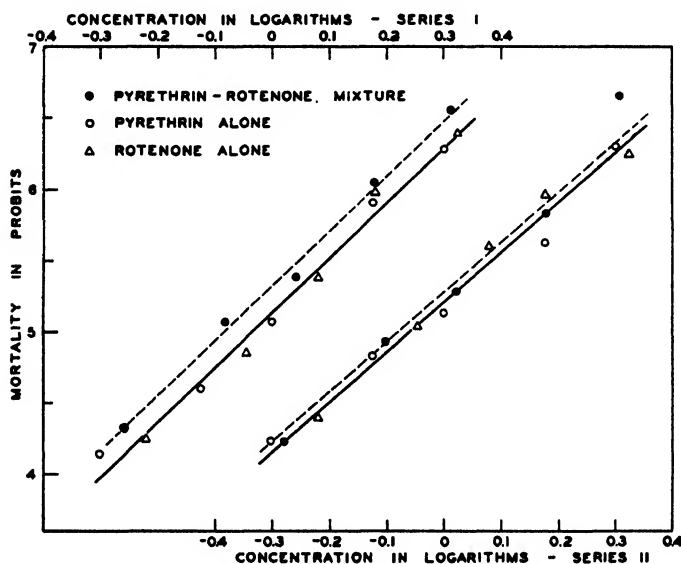
Fig. 6. Dosage-mortality curves for pyrethrin, rotenone and their combinations applied in alcoholic solutions to adult house-flies by a settling mist technique. All concentrations of rotenone have been multiplied by 6·03 to convert them to a pyrethrin standard. Data of Le Pelley & Sullivan (1936).

in 25 times, so that this mixture, containing 84·16 % of pyrethrin, was more toxic than would be expected from the potency of its ingredients. For series II, $M = -0.0184 \pm 0.0232$, a difference which could easily occur by chance, so that this mixture, containing 94·10 % of pyrethrin, showed no increase in toxicity over that expected from equation (5). These percentage compositions were determined from the relative weights of the two active principles in mg. per c.c., and with minor discrepancies were constant within each series. Since the toxicity of solutions containing both ingredients was greater at certain proportions of the two constituents

than could be predicted from the dosage-mortality curves for rotenone and pyrethrin alone, there was definite evidence of synergism, and these mixtures of rotenone and pyrethrin could not be classed under similar joint action as implied originally by Le Pelley & Sullivan (1936) but disputed by H. H. Richardson in a footnote to the original paper.

Synergistic action

Synergism forms a third type of joint action, characterized by a toxicity greater than that predicted from experiments with the isolated constituents. When the constituents of a mixture act independently, its toxicity does not depend upon the relative proportions of the components but only upon their inherent potencies. Synergistic action, in contrast, also involves the ratio of one component to the other. At the seat of action in the organism the two ingredients react with the living tissue as if they formed a single compound of enhanced toxicity. One would expect, therefore, that even if they differ both in potency and in slope when tested apart, the dosage-mortality curve for a given mixture would resemble that for a single poison when plotted in terms of logarithms and probits. No changes in slope would be expected which do not occur in the curves for the separate components. Any more inclusive formulation which will account for variations in the relative proportions of the ingredients must be consistent, therefore, with the basic dosage-mortality curve when the ingredients occur in a fixed ratio.

The opposite of synergism is antagonism, which is characterized by a smaller toxicity than would be expected if the ingredients acted independently. One component interferes with the action of the other. Antagonism has been studied much more extensively than synergism, especially in its relation to pharmacology, and various mechanisms have been proposed to account for the quantitative relations in specific cases, theories which have been reviewed recently by Clark (1937). These are not of such a nature that they can be used in reverse form as an explanation of synergism except in special cases. Judging from the specificity of the relations in drug antagonism, no blanket equation for synergism is likely to hold for all possible ratios between two constituents. Yet within a restricted range, phenomena which probably differ in essential detail and complexity may show useful and striking similarities which invite generalization.

The basic data for a quantitative study of synergism consist of a series of dosage-mortality curves determined under comparable conditions, each curve representing a mixture in which the ingredients occur

in a single fixed proportion. In the simplest case the rectified curves are parallel within the limits of the experimental error and can be fitted with a single combined regression coefficient. The effectiveness of each mixture is determined from the ·equation of its curve in terms of the dosage producing a given mortality, such as the LD50, although the precision is improved if the mortality used for this purpose is the weighted mean probit for the series of curves.

Only two factors now remain, an equally effective dosage for each mixture and its percentage composition in terms of the essential ingredients. Two methods may be proposed for relating these variables. The more direct is to plot in terms of logarithms equitoxic dosages of mixture (the dependent variable) against the percentage in each of the more active ingredient (the independent variable). Although the interrelations of the two ingredients in such a diagram are relatively involved, it preserves the distinction between cause and effect inherent in the design of the experiment, which is an advantage statistically. It is also a more convenient form for later practical use. In several cases this relation has proved to be rectilinear over a useful range of compositions, so that within this range the results could be fitted by the equation

$$\log D_C + i' \log Q' = L, \qquad \qquad \ldots\ldots(8)$$

where D_C is the dosage of mixture $(A + B)$ producing a given kill, Q' is the percentage of the more active ingredient in the mixture, i' is the slope of the line, and L is a constant dependent upon the level of mortality used in fitting the equation. This expression has the further advantage that it can easily be extended to include three constituents or more. Then D_C is the dosage of all three components which is related to the percentages of the two more active ingredients, Q_A' and Q_B', by a partial regression equation

$$\log D_C + i_A \log Q_A' + i_B \log Q_B' = L, \qquad \qquad \ldots\ldots(8a)$$

where i_A and i_B are the partial regression coefficients showing the net relation between the total dose of mixture and the percentage composition of ingredients A and B in logarithms.

The left side of equation (8) may be looked upon as a modified or adjusted expression of dose. For any given mixture $i' \log Q'$ will be constant at all dosages and as a constant added to each $\log D_C$ it merely shifts the dosage-mortality curve for the mixture horizontally without altering the slope. When the dosage-mortality curves for the original mixtures are parallel, this characteristic can be used to test the validity of equation (8) by computing adjusted values for all dosages of each mixture and plotting the observed mortalities in probits against the

logarithms of the adjusted values. All observations in the range from which the adjustment has been derived should fall about a single straight line defined as

$$Y = a' + b \ (\log D_C + i' \log Q'). \qquad \ldots\ldots(9)$$

The regression coefficient b is the combined value determined from the original dosage-mortality curves and a' is computed from the average probit mortality $\bar{y}$ used in solving equation (8) as

$$a' = \bar{y} - bL. \qquad \ldots\ldots(10)$$

The fitted curve represents the effectiveness of any combination within the percentage limits covered by the straight lines of equations (1) and (8).

In original dosage units the relation between the total active material $(A + B)$ and the more active ingredient (A) may be written

$$(A + B) \ A^i = L', \qquad \ldots\ldots(11)$$

where $i = i'/(1 - i')$ and L' is the antilogarithm of $L/(1 - i')$. On this hypothesis the toxicity of a combination of two poisons which act synergistically is determined by the product of their sum multiplied by some power of the more active ingredient. Since the expression provides no lower limit for A, it is undoubtedly restricted to concentrations above a certain minimum. Otherwise a mixture containing only a trace of A would be less toxic than B alone, which is obviously untrue.

An alternative procedure is to plot for equitoxic dosages of each mixture its content of one ingredient against its content of the other. The dose of the combination is multiplied in turn by the proportion of each ingredient to obtain the amounts of A and B which in combination produced a constant effect. When plotted on cross-section paper, preferably in logarithms, each coordinate is restricted to a single component and is independent of the other. Since the relations between them are direct, this mode of analysis is preferred on toxicological grounds although both coordinates are then subject to errors of sampling. If the observations can be fitted by a straight line, its equation has the form

$$\log B + i \log A = \log J, \qquad \ldots\ldots(12)$$

where A and B are dosages of two poisons which in combination have a given toxicity, i is the slope of the line and J is a constant dependent upon the mortality used in fitting the equation. This expression is not equivalent to equation (8), although they may be indistinguishable when one component is always a small fraction of the other. It is restricted to a limited range of dosages for the same reasons as apply to equation (11).

This limitation can be avoided only by including a third parameter in the equation analogous to a minimal dosage for the smaller ingredient.

If A is the more active ingredient whose proportion in certain mixtures approaches zero, then

$$\log (A + K) + i \log B = \log J, \qquad \ldots\ldots(13)$$

where K is a constant which can be determined approximately by assigning various values to K until one is found by trial and error such that $\log (A + K)$ plotted against $\log B$ is a straight line with slope i. The log dose of B alone can be included with the mixtures in solving equation (13). After it has been computed in terms of logarithms, equation (13) can be transformed to

$$(1 + k_1 A) B^i = k_2, \qquad \ldots\ldots(13a)$$

where $k_1 = 1/K$ and $k_2 = J/K$. In this form it is a counterpart to that proposed by Gaddum (1937) for drug antagonism.

Synergism between a phenol and oil. As a numerical example the experiments of Kagy & Richardson (1936) on the toxicity of 2–4 dinitro-6-cyclohexylphenol (component A) and petroleum oil (component B) have been computed with the above equations. Component A, which is practically insoluble in water, was dissolved in the oil in proportions varying from 0·1 to 5 %. These basic solutions or mixtures were emulsified and sprayed on eggs of *Lygaeus kalmii* Stål. in the laboratory at three different concentrations of dilute emulsion. The relevant data are given in the first four columns of Table I. Both the number of eggs exposed and the percentage kill have been corrected for mortality in the untreated controls (4·1 %) by equation (2) or its equivalent.

The first step was to compute the dosage-mortality curve for each mixture. Unfortunately, the authors did not measure the dosage of $A + B$ deposited by each spray but only its concentration in the spray emulsion. Unless the one were directly proportional to the other, the concentration in the spray tank would not be a suitable measure of dosage and when transformed to logarithms would not lead to rectilinear dosage-mortality curves as, indeed, proved to be the case. Cressman & Dawsey (1934) have published data showing that the oil deposited upon camphor leaves was proportional to the logarithm of its concentration in the dilute emulsion, both in field and in laboratory experiments. For laboratory sprays the deposit y in mg. per sq. cm. of leaf surface could be represented by the equation: $y = 0·027 + 0·042 \log x$, where x is the percentage concentration of oil in the spray emulsion. This equation has been "borrowed" to compute the expected dosages of insecticide in mg./100 sq. cm. corresponding to the 1, 2 and 3 % emulsions applied by Kagy & Richardson. When these computed dosages were transformed to logarithms, the probit kill could be plotted against them as a series of

Table I. *Combined action of 2–4 dinitro-6-cyclohexylphenol and petroleum oil sprayed in emulsions against eggs of a plant bug* (Lygaeus kalmii *Stål.); data of Kagy & Richardson* (1936) *analysed as described in the text. Dosages are in mg. of mixture/*100 *sq. cm. of surface*

| Concentration of | | | | | | | | Log LD 50 in combination for component | |
Phenol in oil mixture %	Mixture in spray %	No. of eggs	Net kill %	Log dose x	Probit kill y	b	Log LD 79·6 for mixture	Phenol	Oil
0	1	240	6·5	0·43	3·49	7·16	0·749 ± 0·007	—	0·637
	2	479	40·1	0·60	4·75				
	3	479	58·7	0·67	5·22				
0·1	1	240	9·9	0·43	3·71	7·71	0·694 ± 0·006	− 2·419	0·581
	2	479	59·7	0·60	5·25				
	3	479	72·3	0·67	5·59				
0·5	1	288	30·1	0·43	4·48	7·41	0·617 ± 0·006	− 1·796	0·502
	2	479	73·7	0·60	5·63				
	3	479	90·4	0·67	6·30				
1·0	1	288	58·6	0·43	5·22	7·46	0·511 ± 0·008	− 1·601	0·394
	2	384	94·0	0·60	6·55				
	3	288	97·22	0·67	6·91				
2·0	1	288	81·2	0·43	5·89	6·38	0·431 ± 0·010	− 1·380	0·310
	2	384	97·13	0·60	6·90				
	3	288	99·65	0·67	7·70				
3·0	1	288	86·8	0·43	6·12	8·32	0·388 ± 0·012	− 1·248	0·262
	2	384	99·48	0·60	7·56				
5·0	1	240	96·66	0·43	6·83	—	0·294 ± 0·022	− 1·119	0·160

parallel dosage-mortality curves (Fig. 7) with a combined slope, b_C, equal to $7·392 \pm 0·241$. The combined slope has been used in estimating the log LD50 and other measures for each mixture.

Two alternatives are available at this stage of the analysis, the first relating the dosage of mixture directly to its percentage composition and the second separating each combined dosage into its two components, which are then related to each other. Both methods have been tested. As the basic data for the first, the toxicity of each mixture was measured from its curve in terms of the log dose which killed a specified percentage of eggs. The mortality having the highest precision for this purpose was the weighted mean probit of all mixtures containing from 0·5 to 5·0 % of the phenol component, 5·83 probits or 79·6 %. The log doses corresponding to this value are shown graphically by the intersections of the dosage-mortality curves in Fig. 7 with the lower horizontal line; in Fig. 8, each has been plotted against the percentage (in logarithms) of the phenol component dissolved in the oil. Except for the lowest concentration, the relation of the two variables could be fitted adequately by a straight line. The discontinuity between mixtures containing 0·1 and 0·5 % of A indicates the type of departure which may be expected as the proportion

of the more active ingredient approaches zero. Unless this departure can be shown to have a unique biological significance, such as is postulated for the curves in the first part of the present paper, the line fitted to the larger concentrations by equation (8) should be viewed as a useful approximation. In computing this approximation each observation has been weighted by the reciprocal of its variance, giving

$$\log D_C + 0\cdot307 \log Q' = 0\cdot521.$$

From this equation, the logarithm of the adjusted dose

$$(\log D_C + 0\cdot307 \log Q')$$

has been computed for the individual observations with each mixture.
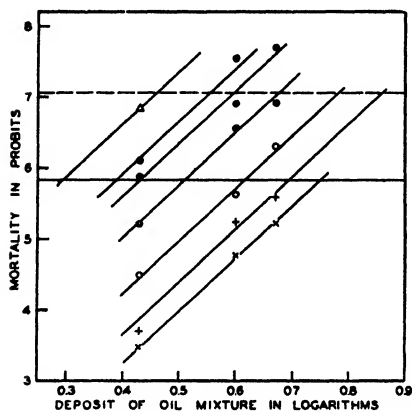
Fig. 7.   Fig. 8.

Fig. 7. Dosage-mortality curves for emulsions of oil containing 0, 0·1, 0·5, 1, 2, 3 and 5 % respectively of a nitro-phenol, reading from below upwards; based on the data of Table 1. Intersection of the curves with the lower horizontal line gives log LD 79·6 (5·83 probits); with the upper horizontal line log LD 98 (7·054 probits).
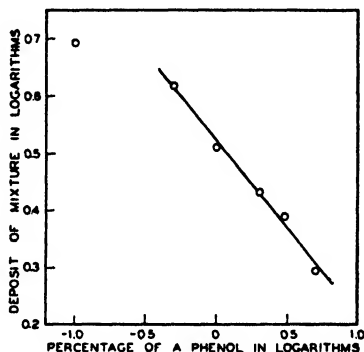
Fig. 8. Relation of dosage giving a mortality of 5·83 probits to the percentage concentration of a nitro-phenol in oil, as determined from the curves in Fig. 7.

When the probit mortalities were plotted against them, all values determined with mixtures containing 0·5 % of A or more fell about the expected curve computed by equation (9):

$$Y = 1\cdot977 + 7\cdot392 \ (\log D_C + 0\cdot307 \log Q').$$

The results are shown in the lower curve of Fig. 9, and the observed mortalities were clearly consistent with the calculated line except as noted.

The relation of the oil deposited to its concentration in the spray has been computed from the evidence of another experiment. The estimated

dosages, therefore, are more or less hypothetical and not convenient for practical use in insect control. This difficulty has been avoided by plotting against the percentage of the phenol component (A) dissolved in the oil the percentage concentration of oil solution ($A+B$) in the dilute spray emulsion that killed 98 % of the eggs (Fig. 10 A). The curve was computed from equation (8), given above, by adjusting the constant L (0·521) to the

higher level of mortality. For 98 % kill, $L = 0\cdot521 + \dfrac{7\cdot054 - 5\cdot830}{7\cdot392} = 0\cdot687$
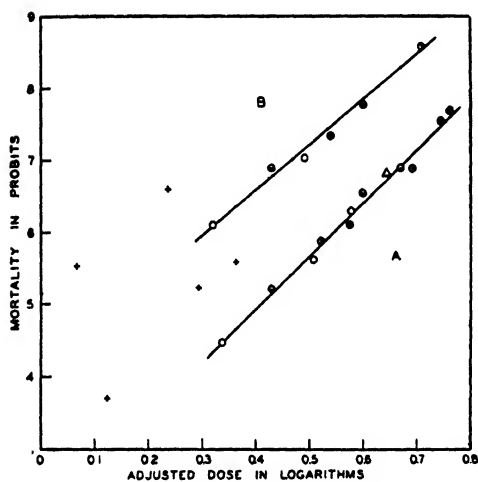
Fig. 9.
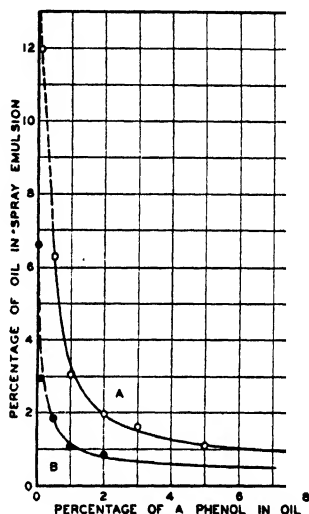
Fig. 10.

Fig. 9. Mortality as a function of the adjusted dose of oil and a nitro-phenol; A, eggs of *Lygaeus kalmii* Stål; B, overwintering *Aspidiotus perniciosus* Comst. The symbols have the same significance as in Fig. 7.

Fig. 10. Concentration of a nitro-phenol and oil required to kill 98 % of eggs of a plant bug (A) and of overwintering San Jose scale (B).

and $\log D_C = 0\cdot687 - 0\cdot307 \log Q'$. The deposits were first computed for values of Q' between 0·5 and 10 % and then transformed to percentages of oil in the dilute emulsion by reversing the equation used in estimating deposits from percentages. The observed values in Fig. 10 represent the intersections of the curves in Fig. 7 with 7·054, the probit for 98 % kill.

⌐ The data have also been analysed by the second procedure, in terms of the separate constituents A and B. For this purpose the LD50 for oil alone and for each mixture was multiplied in turn by the proportion of A and of B to obtain the amount of each ingredient which in combina-

tion killed 50 % of the eggs. In Fig. 11 the phenol component (*A*) has been plotted against the oil (*B*) in terms of logarithms. By omitting the mixture containing 0·1 % of *A* and the value for *B* alone (represented on the abscissa of Fig. 11 as a vertical line), the data agreed reasonably well with equation (12) but these restrictions could be avoided by using its alternative, equation (13). To determine the constant *K*, log (*A* + *K*) was plotted against log *B* for several assumed values varying from 0·02 to 0·04. It was evident on inspection that the relation between the two
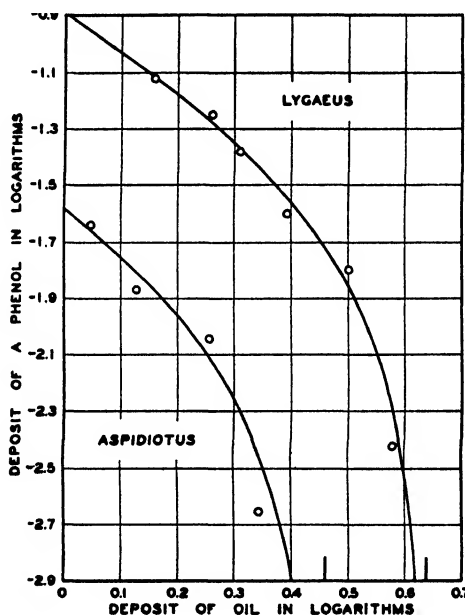
Fig. 11. Relation between dosages of a phenol (*A*) and oil (*B*) which in synergistic combination produced a kill of 50 %. The equation for the upper curve is $(1 + 25·6A) B = 4·29$ and for the lower curve $(1 + 66·7A) B = 2·73$. The vertical lines on the abscissa indicate the toxicity of oil alone, which the curves approach asymptotically.

variables was essentially rectilinear when *K* equalled 0·035 or 0·040. The regression coefficient (*i*) for *K* = 0·035 was 1·07 and for *K* = 0·040 it was 0·98, both near enough to unity that *K* was determined by interpolation as 0·039 so that *i* should be equal to 1·00. Hence the ordinate in Fig. 12 was adjusted to obtain a predetermined slope of 45° or *i* = 1 and the equation of the line drawn through the observations was

$$\log (A + 0·039) + \log B = -0·7764.$$

The straight line of Fig. 12 has been replotted in Fig. 11 after the necessary transformation.

The relation between the two components in units of mg. deposited per 100 sq. cm. of surface reduced to $(1 + 25 \cdot 6A)$ $B = 4 \cdot 29$ for LD50 (equation (13 a)). The same exponent $(i = 1)$ has been obtained in several cases of drug antagonism which have been computed by Clark (1937). If its occurrence is general in synergism as well, an adequate measure of the phenomenon must be sought in the two constants k_1 and k_2. Both k_1 and
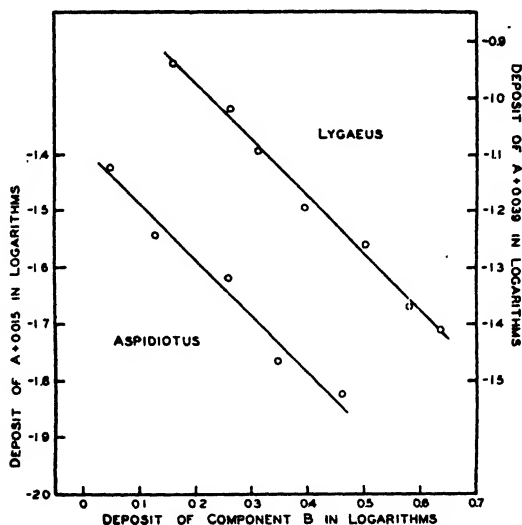
Fig. 12. Diagrams used in computing the equations of the curves in Fig. 11, which on these coordinates are straight lines with a slope equal to 1.

k_2 varied with the level of mortality at which the equation was solved. At the mean probit used in Fig. 8, for example, the above calculation gave $(1 + 19 \cdot 9A)$ $B = 5 \cdot 56$ for dosages killing $5 \cdot 83$ probits. The product $k_1 k_2$, however, was constant; at LD50, $k_1 k_2 = 110 \cdot 0$ and at LD79·6, $k_1 k_2 = 110 \cdot 6$. Any valid measure of the intensity of synergism should be constant at different levels of mortality within the same experiment, a criterion met only by the product $k_1 k_2$, which may be adopted tentatively as a standard measure for synergism analogous to the ratio k_2/k_1 proposed by Gaddum (1937) for the intensity of drug antagonism. Since these mixtures followed the pattern for synergism, it is clear that they could not be described quantitatively in terms of independent action, as was demonstrated by Kagy & Richardson in their original paper (1936).

Kagy and Richardson tested the same materials against the San Jose scale (*Aspidiotus perniciosus* Comstock), although less extensively. When analysed in terms of the total dose of mixture and the percentage of component *A*, results were very similar to those obtained with *Lygaeus* eggs. All mortalities obtained with mixtures containing 0·5 % or more of the phenol component agreed with the expected dosage-mortality curve based on the adjusted dose: $Y = 4·069 + 6·330$ (log $D_C + 0·363$ log Q'). The line determined by this equation has been plotted with the experimental observations in Fig. 9*B*, the mixtures containing 0·1 % of component *A* diverging as before. When compared with its equivalent for *Lygaeus* eggs, the equation for 98 % kill, log $D_C + 0·363$ log $Q' = 0·472$, indicated that the scale was the more susceptible of the two insects, both to the mixture as a whole and specifically to the phenol ingredient. This equation has been transformed to the original experimental units and plotted in Fig. 10*B*.

The results have also been analysed in terms of the separate amounts of *A* and *B* which together killed 50 % of the scales. Agreement with equation (13) was not as close as that obtained with the eggs although the scatter of observations about the line (Fig. 12) was apparently random. The lower curve in Fig. 11 has been plotted from the equation of this line, log $(A + 0·015) + $ log $B = -1·388$. In original units, $(1 + 66·7 \ A)$ $B = 2·73$ and $k_1 k_2 = 182$. The intensity of synergism increased about two-thirds in the San Jose scale over that found with *Lygaeus* eggs for the same materials and technique. This is not unexpected since three factors were involved, two poisons and the tissue of the insect. Any qualitative change in the last of these would be reflected in the quantitative relations of the phenomenon.

Synergism between the constituents of Derris root. Several prominent complex poisons and drugs are natural biological extracts in which the known ingredients may occur in very different proportions. Sometimes the standard procedure described here can be used in determining which of these ingredients have toxic value and the nature of their relation to one another. This can be illustrated by a series of tests reported by Tattersfield & Martin (1935) on the toxicity of Derris extracts to *Aphis rumicis*. Seven samples of root of different origin were extracted with ether, the dried resin dissolved in alcohol and the alcoholic solution diluted with a 0·5 % aqueous solution of saponin for application at various concentrations by a precision laboratory sprayer. The sprays were applied in six series of paired tests, each leading to two dosage-mortality curves based· upon 5–7 concentrations apiece. For the present analysis the

percentage mortality has been determined from the counts of moribund and dead aphids two days after treatment and all curves have been recomputed.

The sensitivity of the aphids varied appreciably from one test to another. The two curves in each pair had the same slope within the limits of sampling error but the slope varied significantly from one pair to another. The level of susceptibility changed similarly between series, so that the four determinations of log LD 50 for sample 5, for example, varied from 1·614 to 1·710, a difference which was significant statistically. For this reason the only comparisons between samples which were not confounded with variations in the susceptibility of the aphids were those within each series. Accordingly the log ratio of potencies (M) and its standard error have been computed for each pair. Using the weighted mean log LD 50 for sample 5 as a standard, the M computed for each of the first four series was added in turn to this standard to obtain the log LD 50 of samples 2, 4, 6 and 7, the standard error of each log LD 50 being equal to the standard error of M, s_M, for the corresponding pair. The log LD 50 for sample 1 was derived similarly from the estimate for sample 7 (series VI) and that for sample 3 from sample 1 (series V). By this procedure the toxicity in terms of the ether extract was determined for each sample on a strictly comparable basis. The results are given in the third column of Table II.

Table II. *Toxicity of ether extracts of Derris root applied as sprays to adult apterous female aphids* (Aphis rumicis); *data of Tattersfield & Martin* (1935) *analysed as described in the text*

Sample no.	No. of dosage-mortality curves	Log LD 50 of ether extract in mg. l.	Percentage composition of extract		Log LD 50 in extract for component	
			Rotenone	Dehydro mixture	Rotenone	Dehydro mixture
1	2	1·397 ± 0·032	27·7	37·7	0·839	0·973
2	1	1·601 ± 0·027	20·7	29·0	0·917	1·064
3	1	1·400 ± 0·050	22·3	43·0	0·749	1·034
4	1	1·640 ± 0·026	13·0	24·8	0·754	1·035
5	4	1·666 ± 0·010	9·8	28·5	0·659	1·121
6	1	1·413 ± 0·042	32·4	29·0	0·923	0·875
7	2	1·422 ± 0·018	35·5	28·0	0·972	0·869

The different toxicities of these ether extracts of Derris were due presumably to variations in their content of specific active principles. The chemical differentiation of these compounds is under active investigation and some of the ingredients which have been isolated chemically and tabulated by Tattersfield & Martin (1935) for these samples may later

prove to be secondary or mixed products having a reduced toxicity.
With this limitation in mind, we may still determine which ingredients
accounted most completely for the observed toxicity. Following the
conclusions of the authors, three components were selected for study:
(1) recrystallized rotenone, (2) dehydro mixture in the residual resin after
removal of the rotenone, which will be treated here as if it were a single
compound, and (3) all other constituents in the ether extract. The
percentage composition of each sample in terms of rotenone and dehydro
compounds respectively is given in the fourth and fifth columns of
Table II; between them they amounted to 38–65 % of the total. To
determine how completely they accounted for the toxicity of the ether
extract, the net relation between the LD 50 for total ether extract and the
percentages of rotenone and dehydro compounds has been computed by
equation (8*a*).

In computing this equation, the log LD 50 for each sample was
weighted by the reciprocal of its variance, so that the variation attributed
to the differences in *A* and *B* as compared with the remaining constituents
and error could be expressed in terms of χ^2. The results are in Table III,

Table III. *Analysis of variance for testing extent to which equation (8a)*
accounts for toxicity of ether extract in terms of recrystallized rotenone
and dehydro mixture. By the present method of computation the sum of
squares follows the χ^2 distribution and the probability integral P has been
computed accordingly

| | All samples included | | | Sample 2 omitted | | |
Variance due to	Degrees of freedom	Sum of squares	P	Degrees of freedom	Sum of squares	P
Rotenone and dehydro components	2	206·06	<0·01	2	214·49	<0·01
All other ingredients and error	4	8·78	0·07	3	0·21	0·98

which shows that nearly all of the toxicity in the ether extract of these
seven samples could be accounted for by the rotenone and the dehydro
components, with the remaining ingredients probably non-toxic. In-
spection of the plotted points showed one aberrant case of low toxicity,
sample 2, and when this was omitted, the toxicity of the ether extract for
the six remaining samples could be accounted for almost completely by
the equation: log $D_C = 2·923 - 0·452 \log Q_A' - 0·556 \log Q_B'$, as shown in
the right side of Table III. The differences between the observed log
LD 50's in Table II and the values estimated from this equation have been

plotted in Fig. 13 as differences from the computed curves for the log per cent of *A* and of *B* respectively. The agreement is all that could be expected with the exception of sample 2.

If the toxicity of these Derris extracts could be expressed in terms of two components, what was their relation to one another? This has been examined by determining the log concentration of *A* and *B* separately in each median lethal dose (Table II) and plotting the one against the other (Fig. 14). Again, sample 2 proved aberrant and when excluded, the remaining observations could be fitted adequately by a straight
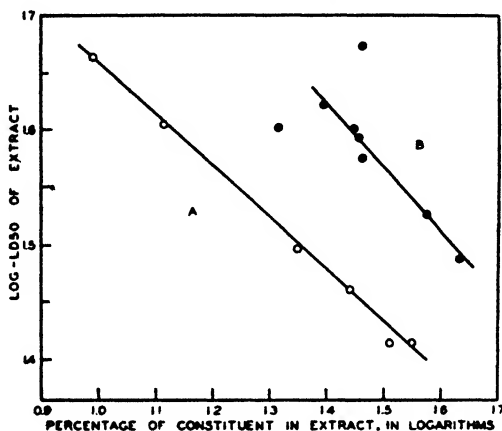
Fig. 13. Relation of median lethal concentration of ether extract of Derris root to its percentage composition of rotenone (*A*) and of dehydro mixture (*B*); data from Table II. Sample 2, represented by the shaded circles, has been omitted in computing the curves.

line (equation (12)): $\log B + 0.820 \log A = 1.660$. When reduced to two essential toxic ingredients occurring in about the same proportions, the toxicity of Derris root could be expressed most easily as a product function of the concentration of the two ingredients. The left side of the equation defines an adjusted dose which for log LD50 averaged 1.660, based on the milligrams of constituent per litre of spray mixture. When computed for each sample, this term varied from 1.632 to 1.667 except for the aberrant sample 2. Sample 2 was considerably less toxic than the others (1.815), indicating that it was out of line not because its full toxicity could not be expressed in terms of rotenone and dehydro mixture but rather because the estimate of this second component contained more inert matter than in the other samples. Rotenone exceeded

dehydro mixture in toxicity since its exponent in the equation $A^{0.82}$ $B = 45.7$ was less than unity.

In contrast with the preceding example, there was no mixture in which one component occurred in a relatively small concentration, so that the relation between constituents could not be tested critically. Any one of several equations could be used equally well in the middle
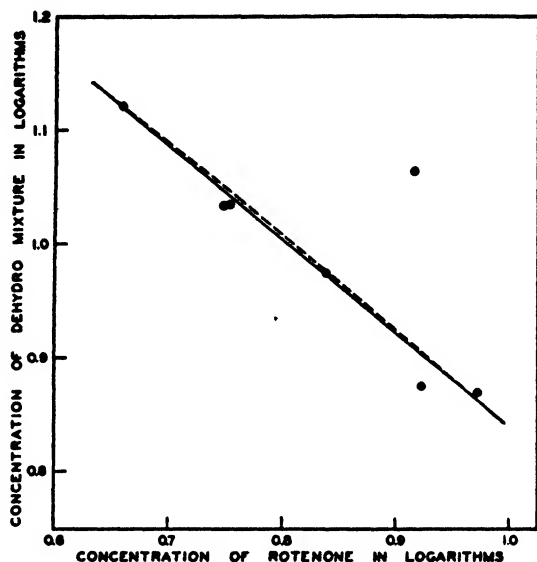
Fig. 14. Relation between concentrations of rotenone (A) and of dehydro mixture (B) which in synergistic combination produced a kill of 50 %. The equation for the straight, solid line is $A^{0.82} B = 45.7$, that for the curved, broken line is $(1 + 0.714 A) B = 56.1$; sample 2, represented by the shaded circle, has been omitted in computing both equations.

range. The equation tested in the preceding example (equation 13)) has been computed with a regression coefficient of 1. Then

$$\log (A + 1.4) + \log B = 1.895,$$

or in the form of equation (13a), $(1 + 0.714A) B = 56.1$. The curve corresponding to this equation has been shown in Fig. 14 as a broken line for comparison with the solid line based upon equation (12). Except for sample 2 (1.903), the adjusted log LD50 based on equation (13a) varied from 1.719 to 1.756 and averaged 1.749. Synergism between rotenone and dehydro mixture was less pronounced than in the preceding example with the constant $k_1 k_2 = 40.1$.

Experiments with fumigants. The toxicity of poisonous mixtures which are ingested, injected or applied by contact sprays involves three factors: dosage, percentage composition and percentage mortality. The extension of these methods to mixed gases and vapours would involve four parameters, since the dosage of an air-borne poison depends upon two factors, concentration and exposure time. Although more experiments have been reported on mixed gases, perhaps, than on any other group of complex insecticides, the data have proved very refractory, largely because the dosage-mortality curve and the LD50 for each mixture have been determined in terms of the exposure time while percentage compositions have been necessarily in terms of relative concentrations. Effective progress in this field seems to depend either upon determining the concentration-time-mortality relation for each mixture, an involved and tedious task, or upon restricting all fumigations to a single standard exposure period and varying only the concentration to obtain the characteristic dosage-mortality curve for each mixture in a series. In the latter case the problem would be essentially the same toxicologically and statistically as the cases which have been described.

SUMMARY

A quantitative analysis of the toxicity of drugs or poisons applied jointly requires that they be administered at several dosages in mixtures containing fixed proportions of the ingredients. From a study of the dosage-mortality curves for several such mixtures, preferably in comparison with equivalent curves for the isolated active ingredients, most cases of combined action can be classified into one of three types:

(1) The first type is that in which the constituents act independently and diversely, so that the toxicity of any combination can be predicted from that of the isolated components and from the association of susceptibilities to the two components. The coefficient of association can be measured experimentally and should be constant at all proportions of the ingredients. When high, the toxicity of the mixture is reduced. The form of the dosage-mortality curve has been examined for several hypothetical mixtures. Whenever the curves for the two constituents were assumed to differ in slope, there was a relatively abrupt bend in the curve for the mixture, the rectilinear segments above and below the break approaching in slope the values for the original constituents. This observation indicates that in homogeneous populations the slope of a dosage-mortality curve is of toxicological significance. Since the same numerical relations would be expected if a single poison were to have two independent lethal

effects within the animal, there is theoretical basis for fitting the linear segments of a dosage-mortality curve separately when a break occurs after transformation to probits and logarithms. This argument has been extended to time-mortality experiments to explain the smoothly concave curves characteristic of natural mortality.

(2) The second type of joint action is that in which the constituents act independently but similarly, so that one ingredient can be substituted at a constant ratio for any proportion of a second without altering the toxicity of the mixture. With homogeneous populations, dosage-mortality curves for the separate ingredients and for all mixtures should be parallel. Although by hypothesis the susceptibility to one ingredient is completely correlated with that to the other, mixtures in this category are more toxic than in the preceding class where association may vary from 0 to 1. The numerical relations have been illustrated by an experiment on the toxicity to the house-fly of solutions containing pyrethrin and rotenone. A mixture with a little less than four equitoxic units of pyrethrin to one of rotenone agreed closely with the definition but one in which the ingredients were about equally balanced showed a significantly greater toxicity than expected on the hypothesis of independent action, indicating the presence of synergism.

(3) Synergism forms the third type of joint action, characterized by a toxicity greater than that predicted from studies on the isolated constituents. It is the reverse of antagonism, which has not been considered directly. Two methods are proposed for the analysis of synergism. The more direct is to relate equitoxic dosages of mixture to its percentage composition in terms of the more active ingredient. When both are in logarithms the relation is linear over a useful range of compositions. This procedure preserves the original structure of the experiment, can be extended readily to three or more ingredients and leads to a convenient practical result. Theoretically it is less satisfactory than a second method in which for equitoxic dosages of each mixture the content of one ingredient (A) is related to the content of the other (B). The equation which satisfies this relation most completely is $(1 + k_1 A) \ B^i = k_2$, where the three constants are computed from the experimental data. When the exponent i is equal to 1, only two constants need be determined and their product, $k_1 k_2$, is proposed as a measure of the intensity of synergism.

The synergism between a nitro-phenol and petroleum oil has been computed by both methods. For mixtures containing from 0·5 to 5 % of the nitrophenol, the deposit of mixture (D_G) killing 98 % of the eggs of a plant bug could be expressed adequately in terms of the percentage of the

phenol (Q') as log $D_C = 0.687 - 0.307$ log Q', for 98 % of overwintering San Jose scale as log $D_C = 0.472 - 0.363$ log Q'. All observations, including those for a 0·1 % mixture and for oil alone which were omitted in the first method, could be fitted satisfactorily in terms of the separate ingredients. For plant bug eggs at LD50, $(1 + 25.6A)$ $B = 4.29$ and for San Jose scale $(1 + 66.7 A)$ $B = 2.73$; in both cases $i = 1$ and the intensity of synergism 110 and 182 respectively.

The full procedure has also been applied to the constituents of seven samples of Derris root. One sample gave an unaccountably low toxicity and was omitted. The log LD50 of ether extract for the remaining six was related to the percentage composition of two components in the extract, rotenone (A) and dehydro mixture (B). Since the toxicity of extract could be expressed almost entirely in terms of these particular two constituents, they were then related to each other by the second method. None of the samples contained a very small proportion of one ingredient, so that several equations were equally applicable, one of them being $(1 + 0.714A)$ $B = 56.1$, from which the intensity of synergism was 40.

The problem of measuring synergism in fumigants has been discussed briefly.

REFERENCES

BLISS, C. I. (1935). *Ann. appl. Biol.* **22**, 134, 307.
—— (1937). *Ann. appl. Biol.* **24**, 815.
—— (1938). *Quart. J. Pharm.* **11**, 192.
BOYCE, A. M. (1931). *Mon. Bull. Calif. Dep. Agric.* **20**, 682.
CLARK, A. J. (1937). *Handb. exp. Pharmak.* **4**. "General Pharmacology." Julius Springer.
CRESSMAN, A. W. & DAWSEY, L. H. (1934). *J. agric. Res.* **49**, 1.
GADDUM, J. H. (1933). *Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 183. His Majesty's Stationery Office.
—— (1937). *J. Physiol.* **89**, 7 P.
HOUGH, W. S. (1934). *J. agric. Res.* **48**, 533.
KAGY, J. F. & RICHARDSON, C. H. (1936). *J. econ. Ent.* **29**, 52.
LE PELLEY, R. H. & SULLIVAN, W. N. (1936). *J. econ. Ent.* **29**, 791.
MURRAY, C. A. (1938). *Soap*, 9, Feb. p. 99.
TATTERSFIELD, F. & MARTIN, J. T. (1935). *Ann. appl. Biol.* **22**, 578.

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

ORDINARY MEETING of the Association held at 2.35 p.m. on Friday, 17 March, in the Metallurgical Lecture Theatre of the Imperial College of Science and Technology, London, the President, Mr C. T. GIMINGHAM, in the Chair.

Discussion on Testing and Diseases of Seeds

The following papers were read:

I. The production, handling, testing and diseases of seeds. By C. C. BRETT, M.A.

II. Notes on seed transmission of *Phoma lingam* in relation to dry rot of swedes in Scotland. By R. W. G. DENNIS, Ph.D.

III. Notes on *Pullularia pullulans* in Ryegrass seed and seed-testing methods as affecting detection of certain seed-borne diseases. By MARY NOBLE, Ph.D.

IV. Hybridization in *Brassicae* and the occasional contamination of seed stocks. By V. McM. DAVEY, Ph.D.

V. Modern methods of seed dressing. By W. R. DILLON WESTON, M.A.

I. THE PRODUCTION, HANDLING, TESTING AND DISEASES OF SEEDS

BY C. C. BRETT, M.A.

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THE title of this paper suggests, at once, a consideration of seed as an article of commerce, and it is from this angle that I propose to approach the subject. The subject is so vast that in the relatively short space of time at our disposal it will be possible to discuss only broad outlines, and attention must be focused principally upon the more important agricultural and horticultural seeds passing through the usual trade channels in this country.

In the production, handling and testing of seeds, is summed up the activities of grower, merchant and seed analyst. In some instances, grower and merchant will merge into one, but for the most part the function of each is distinct, and each is mutually dependent. The final result of the combined activities of these three interests is seen in the normal trade in seeds.

Seed production

For the purpose of this symposium, I propose to discuss production from the economic rather than from the cultural aspect.

In this country, seed production, in its broadest sense, is in part organized and in part almost fortuitous. Organized production is exemplified by a contract system. Under this arrangement the farmer agrees to grow a specified acreage, the merchant supplying the seed and agreeing to take the seed produced from this acreage, provided that it is of good marketable quality. A proportion of the seed of the various cereals, seed of special strains of grasses and clover, most of the root-crop seeds and practically all vegetable seeds are produced in this way.

A direct contract of this kind is not the invariable rule, as sometimes a seed merchant will arrange with another merchant, either in this country or abroad, to place the necessary contract with the grower. In any case, it is usual for the merchant to reserve the right to inspect and rogue the growing crop.

The development, within comparatively recent years, of seed growers' associations, has already begun to influence to a certain extent the general aspect of seed production and marks a step towards organized production of a kind different from that already discussed. The original associations of this kind had as their primary aim the organization of the production of seed of certain local strains of red clover. They aimed at ensuring that purchasers of the seeds certified by them should obtain true and reliable stocks. This necessitated a system of annual crop inspections by competent judges of the strains concerned. Growers joining the associations were entitled to a certificate in respect of each crop examined and approved, and in some cases the association undertook to market the seed certified, whilst in others the members were at liberty to dispose of the seed themselves. More recently still, at least two associations have undertaken to grow certain of the Aberystwyth strains of clovers and grasses.

The Essex Seed Growers' Association is probably the largest, or at least has the widest interests and provides an excellent example of what can be achieved by such an organization. The members of this association now grow for certification, Essex late-flowering and Essex broad red clover and Aberystwyth seeds. As an indication of the growth of this association, it is interesting to note that in 1933 nine crops, totalling 66 acres, of late-flowering red clover were inspected, and that in 1938 the total acreage inspected was 336½, representing 46 crops, actually a slight decrease compared with 1937. Acreages approved and certified in 1938 were 120½ of Essex late-flowering red, 102 of Essex broad red, and 68 of Aberystwyth cocksfoot, timothy, perennial ryegrass and white clover. In the near future, it is anticipated that the association may expect even larger acreages.

Of the other associations, the Montgomery, Hereford, Suffolk and Cornish are at present concerned with local strains of red clover only, and the South-Western association grows Aberystwyth seeds.

The associations already referred to are purely local in operation, and the only scheme of a national character is the Wild White Clover Certification Scheme. This scheme came into operation in 1930 and was prepared by the National Farmer's Union and the Ministry of Agriculture. It is open to all growers of wild white clover in England and Wales and its object is twofold, namely, to assist the grower of suitable seed to obtain a market and a fair price for his produce, and at the same time to

afford a measure of guarantee to the buyer that he is getting the type of seed he requires. Two types of pasture are eligible for recording: (*a*) old pasture, that is, fields that have been under grass for 10 years or more, and (*b*) fields that are "once-grown" from seed saved from old pastures.

Some of these attempts at organized production are still in their early stages, but it is evident that there is a fairly widespread desire to secure to the producer of valuable strains of seeds the differential price that he is entitled to expect for better and more adaptable seeds and to guarantee to the buyer and user that he will get an article well suited to his purpose. It may well be, in the future, that the activities of such associations will exert a considerable influence upon the general trade in seeds.

Unorganized production of seed is dependent upon the open market. In the absence of a prearranged outlet for the seed, the farmer grows a seed crop without reference to the state of the market and not necessarily with a fixed plan for the disposal of the ultimate product. When the seed is threshed, it is offered on the open market and the merchant pays the market price ruling at the time. Generally speaking, seed of all the home-produced clovers and grasses is marketed in this way—with the exception of the special strains already alluded to—and a considerable proportion of the seed of cereals reaches the merchant through this channel. In most seasons there will also be a number of crops of peas and of kale, which, although sown with the primary intention of being used in the green state, may eventually be harvested for seed. In the case of peas, this may be due to an unfavourable market for green peas and in the case of kale to the fact that the keep is ultimately found to be superfluous for one reason or another. From the point of view of general seed production, the practice of saving seed from such crops is not always desirable, as the stocks from which they have been grown may not necessarily be suitable for this particular purpose.

It is clear that under the "contract method", the wholesale seed trade is able to exercise some measure of control over the production of seed. It must, however, be fully appreciated that the maintenance of seed supplies, even under this system, is not a simple matter, especially when it is realized that for certain kinds of seed, if they are sown say in June of 1939, the seed crop will not be taken until the autumn of 1940 and the resulting seed will not be used by the agricultural community until the spring of 1941.

Under the "open-market" system, there is, of course, no control of production. When crops are grown for seed, with no prearranged market, then fluctuations of supply become more pronounced, and in consequence prices are apt to show considerable seasonal variation. The influence of climatic conditions upon prices will often be more pronounced than under the "contract" system.

Certain varieties of some of the root and vegetable seeds are grown regularly in this country for export, and certain seeds are grown abroad on behalf of British merchants for import into and for use in this country. Variations in weather conditions in this and in other countries and fluctuations in the quantities of seed held by seedsmen and therefore in requirements year by year, all exercise important influences upon annual figures of import and export.

Of the seeds normally required for use in the United Kingdom, we are able to produce only a certain proportion of our total requirements of some kinds and none at all of certain others. Seeds which can be and are produced within the United Kingdom include cereals, red clover (both early and late flowering), Dutch and wild white clover,

trefoil, sainfoin, perennial and Italian ryegrass, crested dogstail, mangold, sugar beet, garden beet, swede, turnip, kale, tares, garden pea varieties, broad and runner beans, cabbage, brussels sprouts, parsnip, celery, radish and parsley. We do not, however, produce all that is required for home consumption in every case. In some instances normal home production has always to be augmented by imports, in others, imports—except of special varieties or strains—are negligible, there even being normally sufficient surplus of home-produced seed available for export. Species whose seeds are home produced in only negligible quantities, or not at all, include alsike, lucerne, meadow fescue, the so-called "natural grasses", dwarf-beans and carrot.

Handling of seeds

Cleaning.

Seeds purchased by merchants from growers invariably need further cleaning before they are in a marketable condition, and this has resulted in the production of specialized and often ingenious machines of various kinds, some designed to remove a number of different impurities at one operation, others to deal with only one or two special impurities. A large modern seed-cleaning warehouse is a model of organization and demonstrates clearly the extent of one of the major expenses incurred by those who handle seed in bulk. In the cleaning of seeds, the aim is to remove as much of the contained impurities as possible, without removing uneconomic quantities of the seed itself.

Drying and storing.

Apart from cleaning, there are occasions when seed needs to be conditioned or to be dried before it can be sold or stored. Specialized machinery for the drying of seed in bulk is a necessity in the case of merchants who deal in certain classes of seed. It is especially important that seed to be stored for any length of time should be of a suitable moisture content. For each species there is a limit in this respect, above which seed cannot be stored without incurring the risk of relatively rapid loss of vitality. Successful storage of seed also entails provision for ensuring that losses from vermin and insect attacks are reduced as much as possible.

Dressing.

Many modern seed merchants, especially those who deal in cereal seeds, now make a practice of dressing seed prior to distribution with one of the seed disinfectants now available—usually the proprietary organic mercury dressings. The practice of pre-treating in this way has undoubtedly increased to a considerable extent of recent years, and it is not unlikely that such treatment before distribution will continue to be practised to an increasing extent by seed merchants.

Distribution.

The distribution of seed can be divided into two fairly clearly marked divisions: (*a*) the distribution of home-grown seed, and (*b*) the distribution of imported seed. Home-grown seed, as we have seen, will in some cases have been obtained by the merchants by the "contract" method and in others in the open market. In both cases a certain amount of bulking will often be necessary before distribution to other merchants or to growers. A large proportion of the seed bought in the open market

will, in the case of most kinds of seed, be disposed of locally. Imported seed is chiefly dealt with, in the first place, by a limited number of brokers and wholesale merchants. Distribution is first mainly to other wholesalers and thence to retailers or to growers direct. Some bulks of seed, no matter what the source of origin, will always pass through the hands of a number of merchants before being finally distributed to the consumer, but imported seed usually passes through more hands than does home-grown seed.

The sale of seed and seed analysis

The sale of seed in this country, whether it be from merchant to farmer, or farmer to farmer, is controlled by an Act of Parliament, the Seeds Act 1920, with its comple-mentary regulations. Except in certain specified cases, all transactions involving seeds scheduled under this Act must be in accordance with its provisions, the most important of which is that which states that the seeds concerned may not be sold or offered for sale unless a guarantee as to percentage purity and percentage germination is given at the time of sale or of delivery of the seed. The enforcement of this Act has profoundly influenced the trade in agricultural seeds and now it can be said that sales of seed in this country are primarily upon the basis of known purity and germination capacity. Prior to the introduction of this legislation, a certain proportion of the seed sold throughout the country was, of course, sold upon this basis, but there was no compulsion to do so, and, more frequently, seed passed from merchant to merchant and from merchant to grower at prices fixed chiefly upon the appearance of the seed and not upon any ascertained facts concerning its attributes. With the introduction of control over the sale of seed, there arose, of necessity, a greater interest in seed analysis.

The testing, or analysis of seeds, has developed as the result of attempts over many centuries to determine the value of seed from the point of view of crop produc-tion. The earliest interests in this direction took the form of rites and ceremonies, from which it was expected to obtain forecasts of the success or otherwise likely to attend the sowing of certain seeds or to obtain pre-knowledge of the quality and yield of the crops themselves. From these rites there developed a more precise interest in the seed itself, until in more recent times, following on increased botanical knowledge, there emerged a comprehensive idea of the seed as a living entity.

An an ordered process based on scientific principles, the development of seed analysis dates from about 1869, when Prof. Nobbe was working in Saxony. From then onwards, systems of control of the sale of agricultural seeds were introduced into most European countries, into America and into a number of the Dominions, and, of course, seed-testing stations controlled by the States concerned, or by some official body, were brought into being. For instance, in 1871, the Danish voluntary system of seed control was initiated and finally taken over by the State, with a seed-testing station at Copenhagen. Seed-testing stations were opened at Zurich and Breslau in 1875, at Vienna in 1881, Hamburg in 1891, Wageningen in 1898, Munich in 1902 and Berlin in 1906.

In this country a number of seed firms had started seed-testing laboratories of their own, before there was introduced any state control of the sale of seeds, and a privately owned seed-testing station, open to use by the trade, was started in London, at Wood Green, as early as 1890. In addition, facilities for the testing of seeds were provided at the British Museum, under Dr Carruthers, and the Royal Agricultural Society of

England made arrangements for seeds to be tested for its members by the consulting botanist. In 1910, the United Kingdom Seed Control Station was started by Mr S. F. Armstrong, an unofficial station, started as the result of requests from certain sections of the seed trade, for increased facilities for the testing of seeds.

The first official station in the British Isles was opened at Dublin in 1900—now the Irish Free State Station. The Scottish station was opened in Edinburgh in 1914, on a voluntary basis until legislation was introduced later. The official seed-testing station for England and Wales opened in 1917 in London, later moving to Cambridge, and a station to serve Northern Ireland was opened at Belfast in 1922.

The application of the results of seed analysis to normal trade in seeds was given a tremendous fillip in this country, during the last War. The Board of Agriculture, as it then was, as part of its scheme for furthering war-time food production, introduced a Testing of Seeds Order, which was ultimately consolidated in the Act of Parliament to which we have already referred.

This Act, as was the original Order, was designed primarily to protect the farmer from the danger of unknowingly purchasing and sowing seeds of low vitality and contaminated with noxious weed seeds. No attempt was made to force the farmer to use seed of a better quality than he was prepared to buy; the aim was to make it an obligation on the part of the seller to disclose certain essential facts, so that the purchaser could judge for himself the value of the seed with a fair degree of accuracy. The Act is applicable to all the principal kinds of cereals, grasses, clovers, root and vegetable seeds. It requires that a declaration as to the percentage germination and purity, presence of injurious weed seeds and other specific particulars shall be made in writing to the purchaser at or before the time of sale or delivery of the seed. The introduction of the Act has made it necessary therefore for all dealers in agricultural seeds to ascertain these essential facts concerning seed offered for sale.

The official seed-testing stations in the United Kingdom serve to provide facilities whereby the seed trade may obtain the information concerning seed necessary to enable them to comply with the provisions of this Act. In addition, the Ministry of Agriculture allows the maintenance of private seed-testing stations, by seed firms, but these laboratories may be used by the firms concerned only for the purposes of their own trade. Control of the work of these private licensed stations is maintained by a system of inspection.

In many countries throughout the world, there are restrictions on the sale or import of seed, either enforced by law or adhered to voluntarily, which entail the seller knowing and disclosing facts concerning the seed similar to those required in this country. There have, therefore, arisen in most countries state seed-testing stations, as well as privately owned laboratories run by seed houses, where the chief work performed is the determination of percentage purity and percentage germination of various kinds of seed.

Most seed-testing stations of an official character undertake to determine the moisture content of seed samples, to examine seeds for the presence of seed-borne plant diseases, to make special identification tests of certain difficult species, and to give general advice concerning seed problems.

In practically all countries the determination of the two major features—percentage purity and percentage germination—is carried out along somewhat similar lines, there being certain variations in some directions, due to especial national considerations.

Before discussing methods, it is desirable to emphasize that with respect to the determination of these features, the aim is to ensure that the results of the tests may be utilized with safety as a basis upon which to judge the value of seed, especially in discriminating between different bulks of the same kind of seed. Admittedly, the results of laboratory germination tests do not usually agree with tests made under field conditions and, from the grower's point of view, the criticism that such tests are of little value in assessing the probable germination in the field is valid up to a point. The faithful reproduction of field conditions in the laboratory is impossible of attainment, and therefore methods to determine germination capacity have been evolved which will allow of the conditions imposed being subject to control and also of being standardized. That uniformity and simplicity of method is desirable is apparent in this country, where, in addition to the official stations, there are some thirty-two private licensed stations authorized to test all kinds of seed, and some forty-six others holding licences of limited application. For the purposes of international trade in seeds, the International Seed Testing Association has adopted a comprehensive set of rules, to which all countries subscribing to the Association agree to adhere for the purposes of issuing international certificates.

The determination of the percentage purity has, for practical considerations, to depend upon the examination of a relatively small quantity of seed. This small test sample must be drawn from the bulk sample in such a way as to ensure, as far as possible, that it is truly representative. Various hand methods can be employed and there are a number of mechanical contrivances designed to perform this operation. In this country it is usual to employ the continuous halving method. Two test samples are drawn in this way, and from each is then weighed a definite quantity upon which a purity analysis is conducted. The weights of seed taken vary with different species— the larger the seed, the greater the quantity taken for the purity analysis. Considerable accuracy is desirable at all stages, as the amounts analysed are small and errors will be magnified when the final calculations are made.

Having obtained the weighed quantity of seed, all impurities are separated, leaving the pure seed, of which the sample purports to consist, in a separate heap. This separation is usually performed by placing the test sample upon a suitable glass slab and with a pointed spatula passing the seed in a stream from one side of the slab to the other, all impurities being moved to one side in the process. These impurities are then grouped thus:

Injurious weed seeds. These are species specified in the Seeds Act as being especially noxious.

Other weed seeds. Seeds of weed species other than those in the above group.

Useful species. Seeds of crop plants other than that of which the sample consists.

Inert matter. Obvious material, such as stones, grit, chaff, and vegetable debris; seed so broken or insect-eaten as to be incapable of germinating; light seed of grass species; empty clusters of beet and mangold; immature seed of carrot; sclerotia and spore masses.

The impurities having been identified and grouped, the groups are weighed, the percentage of each group determined and the percentage of pure seed calculated. The results of the two independent purity tests are averaged.

The purity analysis therefore discloses certain features:

(1) The total percentage of pure seed.
(2) The percentage of especially noxious weed seeds.
(3) The percentage of other kinds of weed seeds.
(4) The percentage of other kinds of crop seeds.
(5) The percentage of worthless and harmless material.

In addition it reveals the nature of the individual species within each group, though not the percentage occurrence of each individual. Actually, the Seeds Act necessitates the declaration of certain specified species, where they occur to more than a prescribed percentage, and therefore these species need to be separated and their percentage occurrence determined. In addition, the Act includes a special provision which makes it necessary on the part of the vendor to disclose the presence of dodder, if it occurs to more than a prescribed extent in certain clovers, in timothy and in flax. This entails the examination for the presence of seeds of *Cuscuta* spp. of relatively large quantities of seed.

A scrutiny of the individual species within the groups separated in the purity analysis will sometimes give an indication of the country of origin of the seed, as certain species of plants are characteristic of definite regions.

The purity analysis then gives other useful information, apart from the mere percentage of pure seed. It would be of even more value to the grower if the impurities present in a sample were indicated as representing so many seeds per pound of the sample, but few countries yet insist that this information should be available to the purchaser of seeds.

The purity analysis is by weight, the germination test by numbers, the latter being made upon the pure seed separated in the former. The germination test is devised to find the maximum percentage of seed capable of *complete* germination when placed under suitable controlled conditions.

The general routine in making the germination test is as follows:

A number of separate hundreds of pure seed—the number of hundreds depending upon the nature of the seed—are placed under conditions favourable to germination, and at intervals all seeds which have completely germinated are removed, counted and the number recorded. Intermediate counts of germinated seed are continued throughout the full period of test, but their frequency will vary with different species. In practice they are made as frequently as experience has shown to be desirable, bearing in mind that the seedlings to be counted must be advanced enough for it to be possible to discern clearly whether or not they are quite normal. At the last germination count to be made, the total number of seeds germinated out of each 100 is determined and the average of the sets is struck and this figure taken to represent the percentage germination of the sample. In addition, the number of dead seeds, abnormal seedlings, hard seeds or fresh seeds is noted and the totals checked against the original hundreds.

As a result of experience and of expedience it has been found that it is desirable to fix a maximum limit of time for each species for the germination test, so that it is now generally understood that a germination test shall continue for only a certain number of days, at the end of which time the test will be completed.

The intermediate counting of the number of seeds germinated is undertaken for two reasons:

(1) In order to simplify the actual process.

(2) To give further useful and usually valuable information concerning the seed, in addition to knowing the total percentage of seed capable of complete germination; this information is obtained by noting the percentage of seed germinated at each intermediate count, and this gives an indication of the speed of germination. To the grower such information is especially useful. It is usual to strike an average of the seed so far germinated at the count made at about half-way through the full period of test, and to consider this figure as representing the energy figure of the seed.

For each different kind of seed it has been found desirable to fix a minimum number of hundreds of seed which should be put to germinate, in order to ensure a reliable result. This has been determined as the result of experience, and bearing in mind difficulties of manipulation of material and economy of space. Concerning length of test there is variation between species, as some habitually germinate relatively rapidly, whilst others germinate much more slowly and therefore need a longer period in order to disclose their maximum capabilities.

When the final germination count is made, it is usual to scrutinize the remaining seeds and seedlings, and frequently further information can be obtained at this stage concerning the sample as a whole.

In clover species for instance, there are invariably seeds remaining at the end of the germination test which have not germinated and have not imbibed water. These are known as "hard seeds", and it is possible that in course of time such seeds would imbibe water and germinate, and in fact such is known to be the case, but the rate at which imbibition takes place varies with different species and from sample to sample of the same species, so that in this country it is usual to determine the percentage occurrence of these hard seeds and to report them separately. In other species, too, more especially in brassicas, seeds are encountered which remain ungerminated at the end of the test, but which are not dead. In these cases the seeds are best described as "fresh", and they are usually associated with new crop seed tested soon after harvest. In most cases they are probably seeds which are not fully "germinating ripe", but which in time would fully ripen and so be capable of germinating.

Abnormal seedlings of various types are frequently encountered especially amongst clover species, *Brassica* spp. and onions. It is desirable that seeds which produce seedlings of an abnormal character should not be classed as having germinated, and that for the purpose of determining the percentage germination, only those seeds which produce definitely normal seedlings should be taken into consideration. The criterion of germinability is the capacity of the seed to produce a normal seedling, and a normal seedling must possess a normal root with root hairs, an intact plumular bud and at least one normal cotyledon.

Concerning the types of abnormal seedling referred to, their occurrence can usually be associated with some defect in the seed itself. In the case of clover species, abnormal seedlings, when produced, are most frequently the result of internal injury to the seed, which it has sustained during the usual seed-cleaning processes. Some forms of abnormality can be ascribed to old seed. "Fresh" seeds in brassicas are indicative of recently harvested seed and abnormalities in these species are invariably associated with old seed.

The injurious effect of certain kinds of seed dressings is often reflected in the production of abnormal seedlings during the germination test. In graminaceous seeds, over-dressing with copper sulphate or formalin induces a type of abnormality, in which the radicle is so affected as to produce no primary roots, the plumule only developing. Under certain circumstances, over-dusting with organic mercury compounds results in the production of a particular and characteristic form of abnormal seedling.

The value of the germination test can be summed up as follows:

(1) The total percentage of seed capable of complete germination is disclosed.

(2) The vigour or speed of germination is indicated.

(3) In the case of some species it discloses whether the seed is fully "germinating ripe".

(4) If the seed has suffered physical, internal damage, or damage from chemical seed dressings, such damage is apparent from the germination test.

It has already been stated that to determine the percentage of germination, it is the practice to place the seeds under optimum conditions of growth. It has been found that different species have different requirements in certain directions, in order to obtain satisfactory germination results, so that for each kind of seed there has been evolved a set of conditions to which all samples of the same kind of seed are subjected, in making the germination test. In arriving at these sets of conditions, the aim has been to find a combination which will give the most regular, rapid and complete germination for the majority of samples of the same kind of seed.

For successful germination, under laboratory conditions, the following factors play an important part:

(1) Nature of the seed bed.

(2) Degree of humidity.

(3) Temperature.

(4) Aeration.

All so-called "standard" methods of germination are simply combinations of the first three of these, the differences in methods being reflected in variation of these factors. In practice, the nature of the seed bed is often a vital factor in controlling the degree of humidity, so that the two must be considered in relation to one another.

The actual apparatus commonly in use in making germination tests is probably familiar to everyone, as are the types of seed bed found to be most useful in seed-testing practice. In selecting materials for use as substrata, the chief considerations are water-holding capacity, the ease with which the material can be rendered sterile, and simplicity in manipulation. Sand is the most generally useful material, as it has the obvious advantages of being cheap and easy to sterilize, and enables a large proportion of the total surface area of the seed to be maintained in close contact with the source of moisture. In practice, sand is chiefly used for the larger seeded species, whilst for most small seeds filter paper proves to be most suitable. Humidity and temperature requirements of the different classes of seed cannot now be discussed in detail, but it must be pointed out that for different species variations of these factors are found to be necessary.

We have not the time to discuss methods in detail, nor to consider the finer points

in seed analysis practice, but this brief outline of general principles will serve to indicate the trend of modern practice.

It now remains to consider, very briefly, the interpretation of the results of seed analyses. For the merchant this is relatively simple. He buys and sells upon specific known attributes, such as percentage purity, germination, moisture content, bushel weight, etc.

For the grower the interpretation is somewhat different, as the results of seed analysis give no direct guarantee of productivity in the field. The grower's chief concern being with field results, he must take into account both purity and germination and assess the real value—or percentage of pure germinating seed. In conjunction with price, this figure will afford a reasonable means of comparison. To form some judgement respecting field performance, the speed of germination or energy figure is of most value, and if this figure is known and it is also appreciated that, in general, high laboratory germination suffers less proportional reduction in the field than does a low one, the grower should be in a position to assess the value of any given bulks of seed for comparative purposes. From the detailed information given in the purity analysis, the grower can form a reasonable judgement as to the suitability of a sample for his purpose, bearing in mind the nature and extent of the impurities in relation to the crop seed concerned.

Seed-borne plant diseases

The commercial seed analyst is usually in a position to determine the presence of only those diseases which can be recognized by examination with the unaided eye, but this information, although limited, could be of considerable value to both merchant and grower.

The determination of other diseases is usually outside the scope of the commercial analyst, but it should be within the province of all state seed-testing laboratories to report the presence of those diseases which are known to be seed-borne and of economic importance. There appears to be a growing interest, on the part of those who buy and sell seed, in the general problem of seed-borne diseases, especially in those diseases which can be controlled by the application of the modern seed disinfectants. But the fact that a number of seed-borne diseases can be recognized in seed samples with comparative ease, could be used to greater advantage by merchant and grower than is at present the case. The implication of this fact in the direction of disease control needs no elaboration.

Amongst the seed-borne diseases which may be encountered in commercial seed samples may be mentioned the following:

Tilletia Caries, bunt of wheat—by bunted grains and by adhering spores.

Ustilago Hordei, covered smut of barley—by smutted grain and by adhering spores.

Ustilago spp., smuts of oats—in oat samples—by adhering spores.

Claviceps purpurea, ergot sclerotia in rye, wheat, ryegrass, *Agrostis*, *Holcus* and timothy.

Helminthosporium Avenae, leaf spot of oats.

H. gramineum, leaf stripe of barley.

Tilletia decipiens, in *Agrostis*.

Aplanobacter Rathayi, in cocksfoot.

Septoria Apii, celery leaf spot or blight.
Phoma apiicola, Phoma root rot of celery.
Phoma lingam, dry rot and canker of swede and other brassicas.
Phoma Betae, in mangold and beet.
Ascochyta, pea spot.
Sclerotia of *Typhula,* in red and white clover and sainfoin.
Sclerotia of *Sclerotinia Trifoliorum,* in certain species of Trifolium.

The official stations in the United Kingdom at present report as a matter of routine the presence of only those diseases which can be recognized by the unaided eye, but they are prepared to examine samples for certain specific diseases upon request.

II. NOTES ON SEED TRANSMISSION OF *PHOMA LINGAM* IN RELATION TO DRY ROT OF SWEDES IN SCOTLAND

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THOUGH not a cash crop, swedes form by far the most extensive root crop north of the Border; normally their acreage is about three times that devoted to potatoes. In most districts finger and toe, though present, is not a very serious disease, and the main losses attributable to fungi take the form of dry rot. The particular outbreak which led to the present investigation occurred in the Lothians, but heavy losses are also known to have occurred in the counties of Ross, Inverness, Aberdeen, Forfar, Fife, Perth, Dumbarton, Ayr and Wigtown. Its distribution is, therefore, not to be correlated with the rainfall, since it is at least as severe in the dry north-east as in the wet south-west.

So much work has been done on dry rot in the past that, as regards symptoms, development of the disease and its purely mycological aspects there is little that is new to relate. The chief question on which sharp differences of opinion are still possible is that of the immediate source of infection which starts an outbreak of dry rot in the field. It is well known that once a few mature roots have been attacked infection will spread readily, along the row or in a pit.

Previous workers have shown that *Phoma lingam* can be carried on swede and turnip seed, but with few exceptions they have concluded that such infection is insignificant in amount and plays little or no part in initiating outbreaks of dry rot in the field. According to these authorities the usual sources of such outbreaks are to be sought in infected soil, and in overwintering of the pathogen on infected debris or on susceptible weeds. In 1938 the attention of the Plant Pathology Service for Scotland was drawn to this subject as a result of reports of heavy losses due to dry rot in the swede crops of the Lothians. In this district, where mixed farming is the rule, swedes form the staple winter food for cattle and sheep. The typical Lothian practice is a six-course rotation, including only one Cruciferous crop which may follow either potatoes or oats. There is, therefore, no question of unusually rapid contamination of the soil with infected debris as a result of continuous cropping. On the other hand, the

prevalence of finger and toe indicates that this 5-year interval is not long enough to keep down the population of true soil fungi. In this district dry rot has been recorded on swede crops taken on freshly broken old pasture. It has also been recorded as prevalent where a long rotation amounting to as many as 10 years is practised.

It does not seem likely that the main source of dry rot infection in Scotland can lie in cruciferous weeds. Swedes are used as a cleaning crop and are kept reasonably free of weeds. Also the commonest weeds of arable land in Scotland are Persicaria and spurry. Runch and charlock are seldom seen among swede drills. Infection from hedgerow weeds seems unlikely since the disease appears scattered throughout a field and does not originate in the neighbourhood of hedges.

According to reports of farmers and college officers, dry rot is said to be getting worse each year, and statements have been made to the effect that swede growing in the Lothians will have to be abandoned in favour of some non-cruciferous root such as mangolds. It seems fairly obvious that the very bad season of 1938 was partly responsible for the severe outbreak of disease last autumn. It is the practice in the Lothians not to pit turnips but to cart in a few days' requirements and leave the rest in the field. Hence the crop is subject to excessive frost damage, and at the present time one sees in swede fields at least as much bacterial rot following frost injury as true dry rot. It is probable that the two rots have been confused by farmers and others who have made the extreme statements referred to. I shall be interested to hear members' views on the best way of minimizing spread of dry rot during winter storage.

When a farmer suffers heavy losses from disease there seems to be a natural tendency for him to blame the seed. In view of the numerous complaints received, a number of seed merchants have revived the question of the importance of seed-borne infection and have called for a reinvestigation of the problem.

In preliminary trials swede seeds were plated on malt agar, but this method gave unsatisfactory results owing to the presence of numerous saprophytes. More information was gained by germinating seedlings on damp filter paper in Petri dishes, 100 seeds per dish. Under these conditions *Phoma* infections as high as 2 % were recorded. Infected seedlings rotted and as many as forty pycnidia developed on the decayed radicle. Under these conditions, however, the seedlings were rather weak and it was difficult to maintain uniform humidity. Further experience has shown that the most satisfactory method of estimating infection is that recommended by Buddin, namely, to germinate the seed in the usual way on Jacobsen germinators. Fifteen seeds are as many as can conveniently be accommodated under a 2¼ in. bell jar and, with the germinators used, this implies a total of 840 seeds in each experiment. Even with this fairly wide spacing of the seeds there is still the possibility of disease spreading from an infected seedling to the others on a pad. Hence, in estimating the degree of infection only one infected seedling is counted on each pad, so that the figures to be quoted should be regarded as minima. From Buddin's work it is known that pycnidia of *P. lingam* do not form readily in the dark, but it was not at first realized that full sunlight is essential for reliable results. At first the germinators were set up in a west window, but it has been found that satisfactory results are only obtained when they are moved to a south window and placed so as to receive the maximum amount of direct sunlight. Under these conditions pycnidia may develop in 5 days on the testa of a severely infected seed. Such seeds decay without germinating and pycnidia do not develop on the seedling in less than 12 days. Such seedlings usually develop a rot

which involves radicle, hypocotyl and eventually the cotyledons. Pycnidia form freely on all these organs and may readily be recognized under a hand lens by their copious purple exudate. If the testa is carried up on the cotyledons infection may originate there, as described by Cunningham, but usually the testa is not carried up and infection starts at the base of the hypocotyl or at the root tip. Development of brown markings or stripes on the hypocotyl is not a sufficient indication of infection with *P. lingam.* Such symptoms can be induced by *Alternaria brassicae* and even by *Cladosporium* sp.

Three seed samples of known origin may be quoted to indicate the order of the figures obtained. Of these no. 1 was from a stock of seed other samples of which had given heavily infected crops in 1938. Samples 2 and 3 were daughter seed to be used in 1939. The results are as follows:

Sample 1. Seedlings infected on roots only, 26; other infected seedlings, 8; total, 34 or 4 %.

Sample 2. Roots, 35; others, 14; total, 49 or 5·66 %.

Sample 3. Roots, 13; others, 2; total, 15 or 1·74 %.

As previously mentioned, these figures are arrived at by counting only one infected seedling on each pad. The actual number of infected seedlings present may be taken as about double these figures. It is, of course, arguable that although 5 % of the seedlings may bear *Phoma* spp. with purple exudate this is not sufficient to identify them as *P. lingam.* In admitting the justice of such criticism, one could adduce in self-defence that most modern authorities seem inclined to regard all the *Phoma* spp. with elongated spores about 5–6 by 1·5–2μ, listed on the brassica by early mycologists as falling under the general heading *P. lingam.* The spore measurements of the fungi obtained in our germinators fall within the rather wide range quoted for that species. The *Phoma* spp. present certainly appear to be pathogenic to the seedlings under the conditions prevailing in the germinators and some of them, at least, have yielded cultures which proved capable of initiating dry rot in healthy swede roots. It may be mentioned in passing that two distinct types of growth in pure culture can be obtained from the same pycnidium. The first is dark and rather greenish and spores readily; the second is brown and is almost sterile. Whether these two forms correspond to the mycelial and conidial strains isolated by Hansen from other imperfect fungi it is too early to say.

According to Hughes, 1 % infected seed is equivalent to one infected seed in every 3 ft. of drill. According to Buddin it means the sowing of 3000 infected seeds per acre. Assuming a distance of 2 ft. between the drills the latter estimate implies one infected seed per 2·63 ft. of drill. 5 % infection, therefore, means the sowing of an infected seed to every 7 in. of drill, or about one to every group of seedlings removed at singling. Such a figure is much higher than that recorded as usual in the Ministry of Agriculture's *Bulletin* on dry rot and canker. On the other hand, it is difficult to estimate the probable importance in the field of even 5 or 6 % infection. On the germinator an infected seed either rots or gives rise to an infected seedling which decays and apparently would not develop into an established plant. Moreover, even if infected seedlings became established, the chances are greatly in favour of their being cut down in singling when only one plant is left to each 10 or 12 in. of drill. Hence, the chances are greatly against the dry rot observed on a swede in autumn being due to infection of that particular plant from the seed at the time of germination. On the other hand, it is generally

considered that dry rot originates from infected debris lying in the soil, and it is evident that sowing of infected seed results in distribution of such infection foci very regularly over the soil of a turnip field. If seed-borne infection proves to be important it is probably in this manner that it will be found to be effective.

As regards the question of detecting seed-borne infection in routine seed testing, it appears that few difficulties should arise provided the necessary precautions are observed. The testing can be done in the usual way on Jacobsen germinators exposed to a good light. I understand that in current practice *Brassica* seeds are usually germinated between filter papers in germinators with glass doors. Under such circumstances pycnidial formation is likely to be reduced to a minimum and a very unreliable estimate of infection may result. According to the International Rules the final count is made after 10 days; from the point of view of seed infection this is quite inadequate. Pycnidia do not usually appear in less than 12 days on the seedlings and the samples should be kept for at least three weeks before the final counts are made.

If it is found that seed-borne infection with *Phoma lingam* has a significant effect on the keeping properties of the swede crop, then steps will have to be taken to eliminate it. As previously mentioned, a number of seed firms are already alive to this possibility and have sought advice on the subject. Owing to the thin testa and consequent delicacy of the swede seed, treatment with the standard wet or dry disinfectants is not altogether desirable. Although hot-water treatment may be effective it is hardly practicable at present on a commercial scale. The solution of the problem would seem to lie along the lines of more rigid inspection and selection of seed plants from the point of view of freedom from dry rot and canker.

III. NOTES ON *PULLULARIA PULLULANS* IN RYEGRASS SEED AND SEED-TESTING METHODS AS AFFECTING DETECTION OF CERTAIN SEED-BORNE DISEASES

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THE first record of *Pullularia pullulans* occurring on Ryegrass seed appears to be a note published by Hyde in the *New Zealand Journal of Agriculture* of May 1938. In this note he does not name the fungus but merely states that the fungus causing low germination of Italian Ryegrass in New Zealand is probably the same parasite as is commonly associated with Perennial Ryegrass. In October 1938 in the same *Journal* he published a fuller account under the title "Detecting *Pullularia* infection in Ryegrass seed crops". This is a semi-popular article and in it he states that "it is now generally recognized that the fungus responsible for the low germination of Ryegrass seed is *Pullularia*". The infected seed while within the glumes was indistinguishable from healthy seed but on removing the glumes the kernel was found to be shrunken and rather pink-brown in colour. Infected seed could, however, readily be detected by the use of the diaphanoscope; healthy seed should be bright and translucent but the seed infected with *Pullularia* was found to be dark and opaque. According to Hyde, fresh seed bears masses of pink or buff-coloured spores on and around the kernel. Infected seeds failed to germinate.

In December 1938 our attention was drawn to this question by a firm of seed merchants who had seen the above-mentioned articles and wished to know if the fungus was present on Ryegrass handled in this country. Samples of seed of *Lilium perenne*, and of mixtures of *L. perenne* and *L. Italicum*, were submitted for microscopic examination and *Pullularia* was found easily and readily.

Seeds which were opaque on the diaphanoscope were selected. It was found that the glumes were, in most cases, firmly stuck to the kernels and that these were mostly shrunken as Hyde described. But, where Hyde found masses of spores on the kernels of fresh seeds, we only found on these dried mature seeds little grains of rather powdery consistency, but these seemed to account for the glumes having been so firmly attached to the kernels.

When infected seeds were placed in a drop of water and examined microscopically the spores were seen to float off into the water in very great numbers. They were hyaline and oblong in shape, measuring roughly 12 by $2\,\mu$. These spores failed to germinate either in hanging drops of water or on poured plates of any of the ordinary laboratory media, so that, at first, it was difficult to identify the fungus without having it in culture. Eventually, cultures were obtained from the mycelium present within the seed. These cultures grow very quickly, producing masses of pink or buff-coloured spores on the surface. *Pullularia pullulans*, being synonymous with the old species *Dematium pullulans*, has the property of producing spores which bud in the yeast-like fashion characteristic of the Saccharomycetes, and as the spores described proceeded to bud freely it seemed likely that this fungus was indeed *Pullularia*. So many of the pink spores are formed in the cultures that they very soon have the appearance of a bacterial slime on the surface of the medium. The hyphae are at first hyaline but soon they become opaque with thickened walls and are brown in colour; dark brown chlamydospores are formed more slowly.

All these features agree well with a description of *Dematium pullulans* in culture published by Bennett in *Annals of Applied Biology*, 1928. It seems fairly certain that we are dealing with *Pullularia pullulans*, but whether it is the cause of the death of the Ryegrass seed in this country and in Ireland, as Hyde has found to be the case in New Zealand, has yet to be demonstrated.

A few experiments have been carried out on the germination capacity of infected seeds. It was found that as a working hypothesis it could be assumed that the opaque seeds were infected with *Pullularia* and that most of the translucent seeds were free. It seemed, at first, that all the translucent seeds could be regarded as healthy but, later, it was found that some carried the fungus, although these were not so heavily infected as the opaque seeds. It must be emphasized, however, that when a sample of Ryegrass seed is examined on the diaphanoscope, it is not possible to distinguish at once the translucent and opaque seeds as there is a range from those which are quite light brown to those which are darker brown and so on to those which are almost black. There is a strong human element involved in selecting what are to be termed opaque and translucent seeds, so that any attempt to analyse results statistically is not entirely successful.

In the first experiment fifty opaque seeds were picked out of a sample and put through a germination test along with fifty translucent seeds from the same sample. Of the fifty opaque seeds only two grew and of the translucent forty grew. All the seeds were examined microscopically after the test and it was found that of the

forty-eight seeds which failed to grow forty-five were heavily infected, three were slightly infected, while of the two which did grow one was only slightly infected and the other was free from the fungus. Of the translucent seeds which grew thirty-five were not infected, and of those which did not germinate five were found to be infected.

Through the very kind co-operation of Messrs David Bell Ltd., of Leith, further tests have been carried out on these lines. The germination of samples was determined, then the percentage of opaque seeds in these samples and, finally, independent germination tests of opaque and translucent seeds were carried out. All through these tests mycological checks were made to ensure that the opaque seeds selected were infected and the translucent ones healthy, and it was found that a high degree of accuracy was attained. In most cases the germination capacity of the infected seeds was higher than in the original test but the average is still very low, about 15–17 %. In one test the analyst selected what she termed as "very dark" and "dark" seeds; the former had a germination capacity of 8 % and the latter 40 %. This seems to suggest that the degree of infection is indicated by the degree of opacity. The actual figures obtained in one of the tests were as follows: germination of the sample as a whole 63 %; percentage of opaque seeds in the sample 25 %; germination capacity of the opaque seed 17 %; and of the translucent seed 84 %.

The results of the tests show that there is a correlation between the occurrence of *Pullularia* and reduction in germination capacity of the seed, but it has not yet been proved to be the only cause of the death of the seed. The fungus has now been found in Irish, Ayrshire and Aberdeenshire seed.

One interesting sample of Danish seed was examined. This seed appeared dark in colour and contained many opaque kernels, but when these were tested 93 % grew. However, it was found on examining the seed microscopically, that *Pullularia* was not present and it was concluded that the dark colour was due to some other factor such as weathering. Experiments have also been carried out on the effect of seed dressings. Treatment had no effect on either opaque or translucent seed. On making sections of infected seeds, hyphae were found in a layer running round the periphery of the kernel in the region of the aleurone layer and penetrating the endosperm. In some seeds the scutellum was transformed into a mass of hyphae rather like a sclerotium, while hyphae seemed also to be present in the tissues of the embryo. At several points hyphae had emerged on the surface of the kernel and formed fructifications containing spores.

Some difficulty has been encountered in differentiating the hyphae of *Pullularia* from that of the endophytic fungus described by Miss McLennan as occurring constantly in the seed of *Lolium perenne*. Sections of translucent seeds showed no hyphae so that it seems reasonable to suppose that the hyphae found in the opaque seeds belonged to *Pullularia*.* However, McLennan records the following observation: "When examining a sample of English rye grass from Ireland a specimen was occasionally found showing hyphae invading the starchy endosperm. A careful examination of the aleurone layer of such a grain showed that the hyphae were also running riot here. In addition the scutellum showed an extraordinarily large amount of the fungus. These phenomena were not confined to the sample from Ireland; one of English rye grass from South Africa also contained certain grains showing an extraordinary distribution and growth of the fungus. As before, both the aleurone layer and the scutellum were

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* The sections referred to were cut on the freezing microtome as circumstances did not permit the employment of more definitive technique.

permeated by intracellular hyphae. In one particular case the scutellum, which normally is packed with aleurone grains, appeared to consist of a dense sclerotial-like mass of threads."

Although Miss McLennan does not say so, it seems likely that seeds such as these would not grow. Miss Sampson, working with *Epichloë typhina* on *Festuca*, records instances of the endophyte running riot, such seeds being moribund. A few experiments have been started in an attempt to determine the pathogenicity of *Pullularia* towards *Lolium* spp., but results are not yet available.

In conclusion, mention should be made of the various methods employed by seed-testing stations and their effect on fungi, pathogenic or otherwise, which may be present on the seed.

For example, in the testing of the germinating capacity of cereal seeds, in certain stations it is the practice to plant these vertically with the germ downward in trays of sterilized soil, and projecting about half their length from the soil. If the infection of these seeds with species of *Helminthosporium* or *Fusarium* be considered, it is apparent that placing the seeds in this way tends to suppress the symptoms of infection by these fungi, particularly the twisting and distortion which occurs when such infected seedlings force their way through soil and which give rise to the condition known as pre-emergence blight. The test mentioned is not intended primarily as a test of the sanitary condition of the seed, but it seems that the percentage of abnormal seedlings is reduced owing to the artificial method of planting the seed.

This is a case where the development of the fungal flora is suppressed but, in other instances, the saprophytic fungal flora is artificially accentuated. It is sometimes the practice to germinate seeds of Burnet and Sainfoin between sheets of absorbent paper which, in turn, lie on flannel pads. It was found that such seeds were often smothered by saprophytic fungi such as *Mucor* spp. which did not develop if the seeds were planted in soil. A simple experiment was carried out with these seeds and it was found that seeds dressed with a mild fungicide and then germinated in the pads had the same germination percentage as untreated seeds germinated in soil, and that this figure was higher than that of the untreated seeds germinated in the pads. There is provision for treatment of seeds in the International Rules for Seed Testing where, under the heading of Chemicals, it is stated: "Treatment of the seed with chemical solutions to hasten the germination is not allowed. On the other hand, the treatment of seeds with disinfectants such as are in general agricultural practice to eliminate disease, is permissible", provided that this is mentioned on the International Analysis Report and the germination figure of the untreated seed is shown. Probably this rule was really framed to cover such as the dressing of cereals infected with *Helminthosporium*, but it would be interesting to know whether the elimination of saprophytic fungi could legitimately be included.

Also there are such diseases as those caused by *Ascochyta* and *Mycosphaerella*. Infected seeds, in the dry state, do not always show external symptoms which can be recognized but if the seeds are germinated between layers of blotting paper the fungus emerges and forms pycnidia on the surface of the seed, and in this way the infection is easily recognized. If, however, the seeds are germinated in soil the pycnidia do not form so readily and, even when they do occur, it is much more difficult to distinguish them among the soil clinging to the seedling. On the other hand, if the seedlings are allowed to remain longer in soil than is required for the ordinary germination test, infection could later be recognized by the lesions which would then form.

IV. HYBRIDIZATION IN BRASSICAE AND THE OCCASIONAL CONTAMINATION OF SEED STOCKS

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CROSS-FERTILIZING crop plants require isolation not only to separate their varieties, but also to exclude any other forms which may be sufficiently closely related to hybridize with them. Among the *Brassica* crops grown in Britain, the two mustards, *Brassica nigra* ($N = 8$ chromosomes) and *Sinapis alba* ($N = 12$), are said to be incompatible with one another and with all other forms. The cabbage tribe *Brassica oleracea* ($N = 9$), comprises a wild species and cultivated forms such as cabbage, savoy, brussels-sprouts, kohl-rabi, curly kale, thousand-headed kale and marrow-stem kale. These form all intercross more or less readily and have fertile offspring. A third group within which intercrossing is also possible includes turnips and turnip-like rapes, *B. rapa* ($N = 10$) and swedes, swede-like rapes and certain kales, *B. Napus* ($N = 19$). Until recently it was doubted whether crossing was possible between *B. oleracea* × *B. Rapa* or *B. Napus*, but Roemer (1935) has succeeded in crossing the swede ♀ with several forms of the cabbage tribe. The difficulties of obtaining the cross artificially suggest that there is very little risk of its occurrence in nature. According to U (1935), *B. Napus* is a natural amphidiploid containing genomes of the types constituting *B. oleracea* and *B. Rapa* respectively.

B. Rapa includes white and yellow turnips and two rape forms. These are all interfertile and give fertile hybrids. The turnip-like rapes are not usually grown in Britain, so there is seldom any risk of contamination by these. One is an annual which is grown for oil seed on the Continent, while the other is a biennial, the seed of which is imported under the name of Rübsen or bird seed rape. On occasion this seed has been accidentally sown; it is larger than turnip seed but light red in colour. The plant is worthless as fodder, having turnip-like leaves and a woody root shaped like a malformed sugar beet. The cultivated forms, which have been listed here as *B. Rapa*, are supposed to have been derived from the wild species, *B. campestris*, with which they are compatible. This species is stated by Bentham & Hooker to be a frequent weed of cultivation in Britain. There are also records of colonies which are supposed to have been "escaped" rape forms.

When *B. Rapa* is crossed with *B. Napus* vigorous hybrids are obtained. If one of the parent forms is rape-like, the "bulb" of the hybrid is worthless; but true swede × turnip gives a hybrid which has a very large bulb. This is usually disfigured with "hybrid nodules" and swellings which have been attributed variously to *Bacterium tumifaciens* and other causes. The hybrid has an unbalanced constitution and consequently gives little seed, the offspring being almost invariably monstrosities. Some sort of balance can be regained, however, and the author has bred lines by self-fertilization for six generations. These have remained monstrous in form, but a back-cross in which the hybrid was crossed with swede has given a few lines which have become quite self-fertile and are now fairly good, almost swede-like types. Frandsen & Winge (1932) obtained an amphidiploid, or hexaploid race from their hybridizations of swede and turnip. It is unlikely that contamination by turnip would have any

lasting effect on a stock of swede; the low fertility and striking appearance of the hybrid would lead to its rapid elimination.

The cultivated forms known as *Brassica Napus,* include yellow and white swedes, giant and dwarf rapes and certain kales, which are fully interfertile and yield fertile offspring. The greatest risk to swede stocks comes from the rapes which are widely grown and may be encountered as ground-keepers or as seed in badly cleaned bags or machinery. A common rogue of the swede crop is called the "bastard" or "bulbless bolter". It is most readily observed as a dark green plant standing above the general level of the crop, but not all forms bolt. One sample of green-top swede observed by the writer was highly contaminated with a short bulbless form. This could hardly be detected till the crop was lifted, and it was subsequently found to be identical to dwarf rape. A number of these rogues have been collected and self-fertilized at the Scottish Plant Breeding Station. The progenies were grown alongside authentic samples of rape, and segregating generations of swede × rape. Nearly all the rogues bred true and could be matched with either giant or dwarf rape. As the tallest specimens were usually sent in, there was a somewhat greater tendency for bolting than in average giant rape.

A few of the rogues have proved to be "bastards" in that they behaved like hybrids of swede × rape or like descendants of the cross. Rape has two dominant factors each causing the tissues of the root to be white, and when both are absent the tissues are yellow as in the swede. Flower colour is also connected with these factors, giant rape having a bright lemon yellow, and dwarf rape a dull pale yellow colour. In swede and hybrids with yellow tissues the flower is a dull buff colour. Consequently the rapes, the hybrids and most of the derivatives can be detected by flower colour in a multiplication crop of swede. The swede × rape hybrids can be graded up to have bulbs of large size by repeated crossing with swede and by selection. These bulbs are very stringy and would spoil the quality of a crop, but if high dry-matter content were the basis of selection there would be risk of their inclusion. Occasionally cases of mass-contamination occur and may reach the Law Courts. It is then argued on the one side that the seedsman has failed in his duty and on the other that some form of reversion has occurred. Such a phenomenon has not been observed by the writer. It is possible that in such cases there may be environmental bolting of the whole crop together with some degree of contamination by rape to account for the white roots which have usually been described.

The third set of forms which may be included in *B. Napus,* since they appear to have the same chromosomal complement ($N = 19$), is the rape-kale. There are certain varieties on the market called kales which are quite compatible with the swede. Sutton (1907–8) mentions two such forms, asparagus kale and ragged jack kale. The latter is no longer in cultivation and Sutton's present variety is known as Favourite rape-kale. Fill-gap and hungry-gap kales also belong to this group. A case of contamination recently occurred in Aberdeenshire, where strains adapted to local conditions are propagated by farmers. Plots of swede and fillgap kale were seeded quarter of a mile apart, which for some reason was apparently insufficient isolation, for about 10 % of the swede offspring were hybrid in type, having small bulbs with fang-like roots and curled leaves. The combination of bulb formation and curling of the leaves can be obtained from crossings of this nature, but it appears that genetical linkage may cause repulsion of the more pronounced expressions of these characters.

REFERENCES

(1) FRANSDEN, H. N. & Ö. WINGE (1932). *Hereditas, Lund,* **16.**
(2) ROEMER, W. (1935). *Z. Zücht.,* **20,** 3.
(3) SUTTON, A. W. (1907–8). *J. linn. Soc.* (Bot.), **38.**
(4) U, N. (1935). *Jap. J. Bot.* **7,** 3–4.

V. MODERN METHODS OF SEED DISINFECTION

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THE subject of seed disinfection and seed protectives provides an interesting example of the vagaries of fashion, and if time had permitted I should have liked to quote one or two of the treatments which have been advocated in the past.

It is, however, modern materials and methods with which I must deal, with the wet treatments such as copper sulphate and formalin, and the dry treatments using basic copper carbonate powders and those proprietary dusts which contain organo-mercury compounds as their fungicidal basis.

Copper sulphate is a good fungicide for the control of covered smut of wheat but it has the drawback that it may decrease germination and this reduction in some circumstances may be high; moreover, if not carefully carried out, abnormal seedlings are frequently produced. It is not a suitable fungicide for oats or barley as the vitality of these grains is injured by such treatment. Formalin is the better fungicide and excellent control of the smut diseases—excluding loose smut of wheat and loose smut of barley—can be obtained; moreover, germination is less impaired.

There are several objections to wet treatments. The moistening of seed grain is a troublesome and laborious operation, and it is necessary to dry the grain quickly after treatment and to sow as soon as possible, otherwise germination may be affected and abnormal seedlings result. It is not surprising, therefore, that dry powders have been developed as seed disinfectants. One of the first employed in this country was a finely divided basic copper carbonate which was used at the rate of 2 oz. per Imperial bushel of grain and from 1923 onwards trials with this dust were made by several workers. For the control of bunt of wheat it was moderately effective provided that the contamination of the grain was not heavy, but its vogue in this country was of short duration although it is interesting to recall that its use in Australia is extensive and most satisfactory. Relatively few farmers used this material and their first introductions to dust disinfectants for seed treatment were proprietary articles containing certain salts of mercury as their fungicidal basis.

Formalin, although an effective fungicide for bunt of wheat, covered smut of barley and smut of oats, did not give a satisfactory control of leaf stripe of barley and leaf spot of oats, and very frequently these diseases were responsible for serious losses and were causing much concern to farmers. In the control of these *Helminthosporium* diseases the organo-mercury seed disinfectants were strikingly successful, and in addition gave good control of the covered smuts. In some cases, however, the control of oat smut was not as effective as a formalin treatment, and it is suggested here that future researches might remedy—or perhaps have already remedied—this defect, for the disease is an important one and more prevalent than is generally realized.

It must be admitted that the introduction of these products is an advance of fundamental importance in the history of synthetic fungicides, as a transition from products of specific fungicidal activity to materials of general fungicidal properties has almost been achieved. The credit for this development must be given to interested commercial firms, for it is very largely by their activities that this result has been obtained.

Among some farmers'there is an impression that these dusts are a panacea for all ills to which corn is subject and not infrequently complaints are made that after seed treatment disease has appeared in the crop. Enquiry shows that the diseases in question are loose smut of wheat and leaf blotch of barley. Many observers consider that loose smut of wheat is becoming more prevalent and intense, but it may well be that this apparent increase is connected with the introduction of certain French wheats which appear to be more susceptible to the disease. The grower, not un-naturally, frequently confuses leaf blotch with leaf stripe. Fortunately the former disease cannot be regarded as of serious importance although it probably does affect both the yield and quality of barley, but the seed disinfectant cannot be held at fault as we have no evidence that the disease is seed-borne. Both dusted and undusted seed from a very badly affected barley crop has been sown and perfectly clean seedlings have resulted; indeed, it is not generally until the end of May that we notice the disease at the barley auricles. In the season 1935–6 we tested some experimental dusts and the proprietary organo-mercury products then on the market to see if any control of loose smut in wheat was obtained. As might be expected, no such control resulted; it must, of course, be made quite clear that no manufacturer claims such a control.

It is sometimes argued that the terms seed disinfectant and seed protective are synonymous, but this is not always so. A seed disinfectant such as formalin has an immediate but no lasting value as it cannot confer subsequent protection on the seed. A bunted sample of wheat may be treated in the orthodox way with formalin and if sown immediately a healthy crop will result, but if the sack be roughly handled after treatment or the sample deliberately re-contaminated, and the seed sown, disease will appear in the crop. With a modern product this should not occur as subsequent pro-tection is afforded. A seed disinfectant such as formalin would be of little value in protecting healthy seed from soil organisms, for if conditions for the germination of the seed were adverse then it would be in serious competition with a large and variable soil flora and fauna. In this connexion the treatment of peas may afford an example. In experiments which we have carried out the evidence has indicated that for sowings earlier than March, when encountered, the application of a suitable organo-mercury compound in the form of a dust is likely to result in an increased stand and yield of marketable pods. For sowings later than this there is evidence that the advantages of the application of such a dressing are doubtful.

In what has been said it may have been implied that the only seed protectives are of the organo-mercury type; there are, however, as you are aware, other fungicides such as cuprous oxide which are of value for specific purposes of disease control, or calomel dust which is a protective against specific insect pests. It is, however, with the organo-mercury seed protectives that I shall deal. The composition of these varies with the particular commercial product but the main ingredients of them are very finely divided inert mineral carriers or fillers with which are mixed or impregnated' small quantities of one or more organo-mercury compounds—an anti-dust, sometimes

of an oily character, and a dye are often added. In the majority of cases the fungicidal salt concerned would appear to be a member of the series R Hg X, where R is a hydrocarbon and X an acidic radicle. In some cases mixtures of these salts are used. In this series there is a close relationship between composition and fungicidal power, the toxicity decreasing with the increase of the molecule of R. This, however, must not be interpreted as meaning that dusts which may contain phenyl or tolyl compounds are less effective in disease control than those containing ethyl salts. There is evidence that the most fungicidal salts are the methyl compounds but their very poisonous nature precludes (or may preclude) their use as seed disinfectants or protectives.

A modern commercial seed protective, apart from its general or specific fungicidal power and the control which it gives, or should give, of disease arising from the sowing of seed which is contaminated or superficially infected with disease organisms, must be reasonably safe for the operator to use, taking the usual precautions when dealing with a poison: moreover, possible risks of phytocidal injuries must be minimized. Admittedly these newer dust treatments are very effective in disease control but these newer methods are often criticized on the following counts:

(1) The treatment necessitates the purchase of a machine for treating the grain.

(2) It involves recurrent labour charges for de-sacking, dressing and re-sacking the seed, and the cleansing of sacks which have held treated grain.

(3) The disinfectant dusts are poisonous and consequently precautions must be taken when using them.

The first criticism is one which may be discounted, for there are on the market several relatively inexpensive types of machines which serve their purpose well and the upkeep of which is negligible.

The second criticism cannot be considered a major one as the recurrent labour charges are very similar to those involved in seed treatment with the older liquid preparations. Perhaps the most valid argument is that these dusts contain certain compounds which are poisonous and consequently the usual precautions in dealing with a poison should be observed. It must, however, be realized that approximately 97–99 % of the dust which is used consists of the inert filler or carrier and 1–3 % only—and sometimes less—of the poisonous salt.

Surely if elementary precautions are taken the dusting of the grain should be reasonably safe. When, however, this dusting operation is carried out for very extended periods—as it might be by seed merchants—then the greater element of risk must be considered and all adequate precautions taken.

The manufacturers of these products state that it is advisable to avoid inhaling the dust, and that the nose or mouth should be protected with a cloth or respirator. Also, that some products should not be handled with wet or moist hands and should not be allowed to come into contact with wounds or broken skin since they have a vesicatory action. Further, they state that after the treatment has been carried out, any dressing remaining on the hands should be removed by rinsing thoroughly. Also, dressed seed should not be used for feeding purposes, and bags which have contained dressed seed should be shaken out or washed before being used for carrying fodder.

If the machine used for the purpose of dusting the grain is not adequately dust-proof, or if the compound used is volatile, then it is clear that the operator cannot avoid inhaling some of the disinfectant dust and, consequently, if dust-proof conditions cannot be secured, masks or respirators should be worn. Risk would be further

minimized by carrying out the dusting operation in the open air and not in a closed barn or shed. The practice sometimes adopted of spreading the grain on the barn floor, scattering the powder over this and then turning the grain several times with a shovel is to be condemned for two reasons. The ensuing disease control is less effective, and the operator will inhale the dust particles. If any of the above arguments which are sometimes advanced can be considered valid, an obvious corrective can be suggested and that is a combined seed drill and duster, i.e. a machine that would dust the grain as it was being drilled. Some years ago we devised a machine of this type (Leake & Weston, 1938) and it was used on a commercial farm in Cambridge.

Although the trials indicated that the mechanism gave adequate control for practical commercial purposes, it was thought that it would not give that complete control which would be desired by the grower of seed grain for the market. It should, perhaps, be pointed out that the results were obtained with a mechanism designed merely to test a given principle and that this mechanism is capable of modification to give better results. Apart from any question of toxicity a suitable machine of this kind would minimize labour charges.

There can, I think, be little question that the disinfection of seed is now more general than formerly, and we would expect to find that there had been a general reduction in seed-borne diseases throughout the country. It is difficult to obtain any reliable estimates or statistics on this matter but field experience in East Anglia indicates that such diseases as bunt in wheat, leaf spot of oats and stripe of barley have been very considerably reduced, particularly the *Helminthosporium* diseases. As I thought that this question of the prevalence of disease organisms in seed samples was a matter of much interest, I asked the Director of the Official Seed Testing Station, Mr A. Eastham, if he could supply me with any information. As you may be aware, the routine purity examination of cereal samples at this Station includes noting and reporting the presence of seed-borne diseases, where evidence of contamination can be determined by unaided-eye examination only. Based on these examinations, Mr C. C. Brett has very kindly prepared for me figures showing the percentage of bunted wheat samples received each season during the past 20 years. These estimates are shown in Table I.

This low percentage of infected samples during the past three seasons may have some significance, especially in view of the increased number of samples over those years. Such samples, however, represent but a fraction of the total seed stocks which are sown, and most millers are still all too familiar with bunted wheat. As a further

Table I. *Percentage of bunted wheat samples received each season*

Season	1918–19	1919–20	1920–21	1921–22	1922–23	1923–24	1924–25
Percentage of bunt	5·7	6·5	8·0	33·0	15·7	16·0	14·5
No. of wheat samples examined	3729	1048	1926	2312	1866	1830	2326

Season	1925–26	1926–27	1927–28	1928–29	1929–30	1930–31	1931–32
Percentage of bunt	11·9	5·6	7·3	5·7	4·8	4·6	6·2
No. of wheat samples examined	2340	2423	2685	2538	1782	2142	2741

Season	1932–33	1933–34	1934–35	1935–36	1936–37	1937–38	
Percentage of bunt	5·1	3·9	4·1	2·4	2·7	2·0	
No. of wheat samples examined	4575	4465	4896	5991	7499	8101	

bearing it may be of interest to record that for several years past it has become more difficult to obtain contaminated or infected stocks for experimental purposes, especially samples of oats and barley infected with *Helminthosporium*.

Frequently, the question arises as to which is the best seed protective for a farmer to use. The answer must be given that the products on the market are on a competitive basis and that there is no existing organization in this country whereby such materials can be tested officially.

Another question occasionally asked is, if these products can in any way injure the seed—members of the series *R* Hg *X* have a characteristic phytocidal effect when applied in overdoses. The seed commences to germinate and the coleoptile appears, but develops abnormally and swells. Root development is restricted in varying degrees. These "aborted" seedlings seldom develop further and do not rapidly decay. Some instances of this type of injury have been noted in practice but they are rare and the conditions under which it has taken place are obscure.

It has already been mentioned that the organo-mercury protectives give no control of loose smut of wheat and that this disease is very prevalent although not very intense. Have modern researches resulted in any effective and practical treatments? In India, Prof. Jai Chand Luthra (1934) obtained a complete control in many cases and in a very simple way. He pre-soaked samples of smutted wheat in water at ordinary temperature for 4 hr., i.e. 8 a.m. to 12 noon; they were then taken out and exposed to the sun from 12 noon till 4 p.m. The crop raised from the treated seed was quite free from the disease, while the control plots of untreated seed had as much as 14 % of smutted heads. It is clear, I think, that such a treatment would have no success here.

In East Anglia we do not advise farmers to carry out the hot-water treatment for the control of loose smut of wheat as the risk of destroying the vitality of the grain is so great, but it is interesting to record that some seed merchants are now experimenting with hot-water treatments.

I have dealt almost entirely with the cereal diseases, but the beneficial vogue of seed treatment is not confined to these crops alone, for by these or other methods a wide range of seeds are now often treated prior to sowing. But is this a modern conception? Peters (1771) in *Winter Riches* says: "Shall I stand alone convicted of all mankind, stand a culprit before my great superiors, when I affirm the great omission in not reminding the farmer, that it is absolutely as necessary for him to take the same precautions, care, and assiduity through every species of grain he commits to the benevolent earth, as to wheat," and concluded, "Is it because they are not of equal value—too vague a word to answer? Let me therefore recommend that duty (you owe it to yourselves) as much to one, as the other; they are all fed with the same food, and nourished in the same earthly bowels...."

REFERENCES

LEAKE, H. M. & WESTON, W. A. R. DILLON (1938). Combined seed drill and duster. *J. Minist. Agric.* **45**, 344.
LUTHRA, JAI CHAND (1934). Some experiments on the control of loose smut, *Ustilago Tritici* (Pers.) Jens. of wheat. *Indian J. agric. Sci.* **4** (1).
——— (1934). The loose smut disease of wheat (vernacular "Kangiari") and some new methods of its control. *Agric. Live-Stk, India,* **4** (5).
PETERS, M. (1771). *Winter Riches,* p. 137.

REVIEWS

Practical Lawn Craft. By R. B. Dawson. Foreword by R. G. Stapledon. Pp. 300 + 32 illustrations and 3 text-figures. London: Crosby Lockwood and Son, Ltd. 1939. 15*s*. 0*d*.

The author of this volume is the Director to the Board of Greenkeeping Research, St Ives Research Station, Bingley, Yorkshire. He has held this post since the establishment of the Station in 1929, and in his capacity as Director, has carried out a vast number of experiments on all kinds of turf problems and has had ample opportunity of examining sports turf in all parts of Great Britain and many parts of the Continent. This book embodies the results of these experiments, and includes also an account of the work of other investigators of turf problems.

It is divided into five sections dealing respectively with the historical aspect of lawns, the formation of new lawns, the maintenance of established turf, turf for sport and other purposes, and turf upkeep in other countries. Detailed instructions are given for the construction of new lawns from sods and from seeds mixtures, and the author emphasizes the superiority of simple seeds mixtures, pointing out that "on swards consisting of the finest turf, bent, or a perfect blend of bent and fescue, may account for almost 100 % of the herbage". The maintenance of established turf is treated from every angle, including the choice of a mower, the practical use of fertilizers, eradication of weeds and earthworms, and the various mechanical operations necessary for turf upkeep. Four chapters are devoted to instructions for the management and upkeep of turf for all kinds of sport from bowls to polo, and a whole chapter is concerned with the establishment and upkeep of aerodromes. The whole subject of lawn craft is treated from the scientific point of view, but in such a manner that it can readily be understood and made use of by the practical man. Details of the cost of the various operations necessary for turf upkeep are included, and instructions for mixing fertilizers and for laying out sports grounds of all kinds are given in the appendix.

This book is indispensable for all professional greenkeepers and groundsmen, and for anyone who desires to be the owner of a good lawn. ADELA G. ERITH.

An Introduction to Industrial Mycology. By G. Smith. Pp. xii + 302. London: Edward Arnold and Co., Ltd. 1938. 16*s*. 0*d*.

This is not a textbook of fungi: it deals with a very heterogeneous series of common moulds and is meant primarily for industrial chemists lacking mycological training. Industrial microbiologists possess, in nearly all cases, a chemical background rather than a botanical one, but there should be ample scope in this field for botanists specializing in microbiology, although they would need to acquire also a good knowledge of biochemistry, and such students will find this book very useful.

Ch. I is introductory, Ch. II deals briefly with terminology and classification, Ch. III is devoted to the Zygomycetes, Ch. IV to the Ascomycetes, Chs. V and VI to the Fungi Imperfecti, Ch. VII to *Aspergillus* and Ch. VIII to *Penicillium* and related species. Later chapters deal respectively with laboratory equipment and technique, the physiology of mould fungi, the maintenance of a culture collection, the control of mould growth, and the industrial uses of fungi. The final chapter is a brief annotated guide to mycological literature, and the book closes with a good index. There is a commendatory foreword by Prof. H. Raistrick, each chapter terminates with a useful selected bibliography, and several chapters contain dichotomous keys. The selection of material is artificial, being determined by the prevalence and importance of particular genera and species in industry either as contaminations or in relation to various processes. The author's treatment is conditioned by the same factors, the major emphasis being laid upon identification and practical considerations. As these aspects of fungal study are largely or entirely neglected in textbooks of fungi this book forms valuable accessory reading.

The work is extremely well done, the chapters dealing with *Penicillium* and *Aspergillus* being particularly useful. The chapter on laboratory equipment and technique is excellent but note might have been made of the cheapness and usefulness of plain medical flats for large size and stock cultures, and also of the manifold uses of cellophane in culture work. The chapters on the physiology of mould fungi and the industrial uses of fungi might, with advantage, have been made much fuller, and in that on the control of mould growth attention might have been drawn to the use of nipagin. There are occasional misprints and, here and there, sentences requiring modification, e.g. p. 62, "Conidial forms known as *Botrytis* are known to belong to several different genera of Ascomycetes"; p. 228, "Many workers prefer to make cultures which are obtained by germination of a single spore, this being an absolute guarantee of purity".

A notable feature of the book are the 127 beautiful photomicrographs arranged on 64 plates and containing some of the best illustrations of micro-fungi yet published. Although only an introduction to the subject, the book is a first class piece of work and bears the hallmark of research experience. Not only industrial chemists but microbiologists, plant pathologists, and botanists will find it of value.   W. B. BRIERLEY.

Cattle Fodder and Human Nutrition: with special reference to Biological Nitrogen Fixation. By ARTTURI I. VIRTANEN. Pp. 108. Cambridge: University Press. 1938. 7s. 6d.

In 1937 the author, who is the Director of the Biochemical Institute, Helsinki, gave three lectures at London University, and one at Reading University and these, together with a brief survey co-ordinating the material, are here reprinted. Prof. Virtanen's researches have, in recent years, fundamentally changed our conception of the nitrogen fixation process by root nodule bacteria, and of the way in which the nitrogen becomes utilizable by leguminous and other plants, although certain of his results have not obtained confirmation on repetition of the experiments by workers in other countries. The first two lectures deal with this subject and are entitled "The

Mechanism of the Biological N-Fixation" and "The Symbiosis of the Leguminous Plants with the Legume Bacteria".

The last two lectures deal with "The Production of Vitamins in Agriculture, with Special Reference to Human Nutrition", and "The A.I.V. Method for the Preservation of Fresh Fodder and its Importance in Agriculture". At first glance these subjects seem to bear little relation to those dealt with in Lectures I and II, but the researches on which they are based arose directly out of the author's investigations on leguminous plants. Lecture III has particular reference to conditions in Finland where winter fruits and vegetables are not easy to obtain, but has also considerably wider application. The lack of vitamin A in the winter rations of cattle in Finland led Prof. Virtanen to seek a method whereby fresh, young, digestible and protein-rich fodder could be made available for this purpose, and this eventuated in his well known A.I.V. silage process, the principle of which is that the pH of the fodder mass must be below pH 4 in order to guarantee a successful conservation. The adoption throughout Finland of the A.I.V. method has solved the vitamin problem in that country, but the process is also of the utmost value in many other regions. Prof. Virtanen is of opinion "that the A.I.V. method will replace, to a great extent, the earlier method of haymaking" and that "the artificial drying of grass, by means of which the same results are obtained as by the A.I.V. process, has no economic possibilities, at least in its present form, of gaining ground in the preservation of fodder".

The author's concluding "General Survey" occupies only a little over three pages, but it epitomizes in a remarkable way the matter and viewpoints elaborated in the previous 100 pages, and it is of first class interest. As much of Prof. Virtanen's original work is published in rather inaccessible Continental journals it is very useful to have this authoritative and up-to-date account in so convenient a form.

<div align="right">W. B. BRIERLEY.</div>

Coffee in Kenya. By the Staff of the Scott Agricultural Laboratories and the Agricultural Economist, Department of Agriculture, Kenya, with a contribution by the Director of the British East African Meteorological Service. Edited by J. McDONALD. Pp. vi + 210. Plates 26. Nairobi: Government Printer. 1938. 5s. 0d.

In his foreword, Mr H. B. Waters, Director of Agriculture in Kenya, states that "This publication is not to be regarded as a textbook on coffee which will serve to teach planters the A.B.C. of coffee cultivation, but as offering to planters some elucidation of problems met with in Kenya plantations". It contains six parts as follows: I, The climate of the coffee areas of Kenya, by A. Walter (pp. 18); II. Coffee soils and their treatment, by G. H. Gethin Jones (pp. 37); III, Cultural practice and factory treatment, by A. D. le Poer Trench, S. Gillett, and T. L. McClelland (pp. 54); IV, Insect pests of coffee, by F. B. Notley (pp. 36); V, Diseases of coffee, by J. McDonald (pp. 43); VI, Economics, by V. Liversage (pp. 13); The book contains numerous diagrams and tables and concludes with an index. The text is clearly and interestingly written although, in the foreword, there is a curious mistake in the dating of the Great War and in Part III the author uses the rather obsolete adjective "fungoid".

The book should serve its purpose extremely well and, although it relates specifically to Kenya, it should have a wider appeal since it has something of the nature of a preliminary monograph on the bionomics of *Coffea arabica*, and it is a useful supplement to its editor's *Coffee Growing, with Special Reference to East Africa*, published in 1930. Research on coffee in Kenya only began about 1924–6 and this publication is a most creditable record of what has been achieved in the intervening period.

W. B. BRIERLEY.

Third Conference on Cotton Growing Problems: Report and Summary of Proceedings. Pp. 168. Empire Cotton Growing Corporation, King's Buildings, Dean Stanley St., London, S.W. 1. 1938. 2*s.* 6*d.*

The Conference was held in London on 22 and 23 September 1938, and was attended by scientific workers representing all cotton growing regions of the Empire. The Report contains a summarized introduction of each paper by the author together with the discussion thereon, and the contributions are grouped under the following heads: A, The place of cotton in agricultural systems; B, Local problems of cotton growing; C, The manuring and cultivation of cotton; D, Some problems other than manuring; E, Diseases and pests of cotton, (1) general, (2) food supply as a factor in insect attack outbreaks, (3) dormancy in insects and its relation to survival; F, Fibre properties of cotton. Two more general papers which do not fit into the above scheme are intercalated, viz. "Systematic observations and studies of plant development in field plots of cotton, and in replicated field experiments on cultivations, manuring and other matters", and "Some problems in genetics whose solution would help the plant breeders".

The width of experience represented in the contributions makes them especially interesting, and the Report is a valuable summary of present knowledge of the particular problems at issue.

W. B. BRIERLEY.

Plant Injection for Diagnostic and Curative Purposes. By W. A. ROACH. Technical Communication No. 10. Pp. 78. East Malling: Imperial Bureau of Horticulture and Plantation Crops. 1938. 5*s.* 0*d.*

Dr Roach has made this subject peculiarly his own and in this Bulletin gives a clear account of his ingenious and fruitful researches. Following an interesting introduction the author discusses fully and critically the methods of plant injection. This section, which forms the bulk of the work, is a masterly piece of writing and is beautifully illustrated. It is followed by brief sections concerned with the determination of the distribution of injected liquids, damage to foliage resulting from injection, dosage, localization of effects of injection, injection of plants when leafless, solid injection, and an all too brief consideration of the general application of plant injection methods. The Bulletin concludes with a short general summary and a useful bibliography of 162 citations.

For a long time botanists have, in a desultory sort of way, been interested in plant injection since on *a priori* grounds it ought to be a valuable and illuminating method of research. On the other hand, the use made of it and the results accruing have never seemed to fulfil its inherent promise. Much of the importance of Dr Roach's work, as Prof. V. H. Blackman points out in his foreword, resides in the fact that he has developed plant injection "into a precise technique which can be followed by others; he has thus placed in our hands a new instrument of great value in both pure and applied biology".

W. B. BRIERLEY.

Plant Physiology. By E. C. MILLER. 2nd edition. Pp. xxxi + 1201. London: McGraw-Hill Publishing Co., Ltd. 1939. 45s. 0d.

In the new edition of this well-known treatise the general framework and chapter headings remain unaltered, but the treatment of various topics has been revised or amplified, and certain new topics have been added. Even with extensive use of small type the inclusion of the new material has necessitated an increase of 300 pages in the size of the volume. There are two new text-figures, the questions concluding each chapter in the first edition have advisably been omitted, and the number of bibliographic citations has been more than doubled. The author's general aims and points of view have not changed, his consideration is strictly physiological in so far as physiology can be separated from biochemistry, and as befits a book by a Professor in an agricultural institution the work has a slight but welcome agricultural bouquet. As in the first edition the text is restricted to the physiology of the green plant and such subjects as microbial physiology, or pathological physiology, except in so far as other nutritional troubles are concerned, are excluded.

During the period elapsing between the editions advance has been made in all directions but more especially, perhaps, in knowledge of the plant cell, transpiration relationships, carbohydrate formation, nitrogen metabolism, translocation, and respiration, and notably in the extension of our knowledge concerning the elements absorbed by plants, and in our understanding of the process of growth in plants. For example, in relation to growth processes, the whole subject of phasic development with its far-reaching theoretical implications, and its practical significance in the vernalization of crops, is essentially a development of this decade. Also, although the existence of growth-promoting and growth-inhibiting substances has been known for some 20 years, all our understanding of them and of their fundamental importance has come during the last 10 years. Similarly, during this period there has been a great extension of our knowledge concerning the role of the minor elements in plant nutrition. All these advances have been incorporated very skilfully by Prof. Miller in his book which is densely packed with information, but since the new edition has developed from the old by transformation rather than by apposition or intussusception, and because the more technical and detailed matter is relegated to small type, the book still retains its readable quality. Some of the new developments are not so straightforward as the older classical material and are, on occasion, subject to controversy, but the author has succeeded in being impartial in his presentation of data and viewpoints.

During the last 10–15 years numerous plant physiological works in English have been published. When the first edition of this book appeared in 1931 it was at once recognized as a contribution of outstanding merit, and it has successfully stood the test of time and usage. The second edition is even better than the first, and as a source-book of information and a guide to the literature of the physiology of the green plant it has no compeer.   w. b. brierley.

Principles of Genetics. By E. W. SINNOTT and L. C. DUNN. 3rd edition. Pp. xiv + 408. London: McGraw-Hill Publishing Co., Ltd. 1939. 21s. 0d.

The two authors are Columbia University Professors of Botany and Zoology respectively, and they form an ideal combination. Their book has long been recognized as one of the best general introductions to the subject, and the third edition will maintain this reputation. The new data and viewpoints developed since 1932 have been incorporated and, although modern genetics is far from being an easy subject, the authors have succeeded in giving a balanced, accurate, and up-to-date picture of the situation in readable and interesting language. The new data largely concern gene locations and chromosome changes, but the discussions of cytoplasmic inheritance, heterosis, multiple factor inheritance, mutation frequency, and polyploidy have been amplified. By condensation of matter, and redistribution of the "Problems" and the statistical material, the new edition has been shortened by 33 pages. Many of the illustrations of the earlier book have been omitted or replaced by better figures, and the chapter bibliographies have been brought up to date.

Any student working through this book and tackling the "Problems" seriously will acquire a very sound introductory knowledge of genetics.   w. b. brierley.

The Genetics of Garden Plants. By M. B. CRANE and W. J. C. LAWRENCE. 2nd edition. Pp. 21 + 287, figs. 62 and 43 tables. London: Macmillan and Co., Ltd. 1938. 12s. 6d.

The first edition of this work appeared in 1934 and received notice in the *Annals* (1935), **22**, 437. The general plan of the book and the mode of presenting material remain the same, but in every chapter there have been considerable re-writing and addition of new data and points of view to bring the work up to date. The main structural change is the addition of a new and valuable chapter on the chemical and genetical basis of flower colour, in which is incorporated an account of *Dahlia variabilis* which, in the first edition, formed part of the chapter dealing with flowering and ornamental plants. This new chapter is, perhaps, the best simple account of this difficult subject available. Also, in the new edition there is a useful appendix listing the chromosome numbers of plants mentioned in the text, and the bibliography has been considerably extended.

A reader familiar with the groundwork of genetics will find the first three chapters an excellent summary of genetical principles, and the remaining chapters a first class account of their exemplification in horticultural plants.

                                                                       w. b. brierley.

The Cultivation of Succulents. By H. JACOBSEN. Authorized translation by VERA HIGGINS. Pp. 106. 8 figs. London: Williams and Norgate, Ltd. 1939. 5*s.* net.

Anyone interested in the succulent plants is familiar with Jacobsen's monograph *Succulent Plants.* In this botanical details of the species were given but only occasional notes on the cultivation of those requiring special treatment. The present volume is to be regarded as supplementary to that work and gives full details of the methods of propagation, the uses and the cultivation in pots and in the open of this type of plant. Interesting chapters are also included on the distribution in nature of succulents and the methods of collection and importation. The translator, herself a well-known authority on the subject and author of several books on cacti, is to be congratulated on making available to the increasing number of enthusiasts in this country a book which contains so much detailed information in so small a compass.   R. H. STOUGHTON.

Research and Statistical Methodology: Books and Reviews, 1933–8. Edited by O. K. BUROS. Pp. vi + 100. New Brunswick: Rutgers University Press. 1938. $1.25.

Dr Buros has collected and classified the titles of "practically all the research and statistical methodology books published between 1 January 1933 and 15 November 1938 and written in the English language. For each book, full bibliographic information is given including both American and English prices and publishers. Hundreds of journals in numerous fields were searched for reviews of these books. The most critical portions of the reviews were then excerpted and collated as published herein." For example, B. 700 is a citation of Snedecor's *Statistical Methods* with details of publisher, price, etc., followed by excerpts from reviews published in ten scientific journals, in each case citing the issue of the journal, and naming the reviewer.

It is an unusual sort of publication but decidedly useful to librarians and others desiring to know scientific opinion on books before purchasing them; continued as periodic issues the series will be of real value as a source of reference.

    W. B. BRIERLEY.

Methods and Material for Teaching Biological Sciences. By D. F. MILLER and G. W. BLAYDES. Pp. xii + 435. London: McGraw-Hill Publishing Co., Ltd. 1938. 21*s.* 0*d.*

This book is intended for teachers of elementary biology and for teachers in training. Part I, "Principles and Classroom Methods", is designed primarily as a text for classes in special methods and deals with the biological basis of education, the objectives of teaching in biological subjects, types of courses, methods of presentation, making a teaching plan, an evaluation programme, the lack of materials and equipment, visual education, how to choose a text, and trends in the curriculum. Part II,

"Preparation and Uses of Classroom Materials", deals more practically with examples of student projects, collecting, culturing and preserving of material, laboratory aids and substitutes, microscopical preparations, and methods and materials suitable for classroom demonstrations and experiments dealing with photosynthesis, digestion, nutrition, and growth, diffusion, circulation, respiration, water relations of plants, the response of organisms, reproduction, and heredity. The book is illustrated by 146 text-figures, contains numerous test questions and useful bibliographies, and concludes with an index.

The book has particular reference to American conditions and problems but much of it is of quite general application. Part I is interesting and suggestive, although here and there a little trite. Part II, occupying two-thirds of the volume, will be useful to all teachers of elementary biology since it is packed with data and practical suggestions concerning the materials and methods of laboratory preparation and teaching.

W. B. BRIERLEY.

THE HISTOLOGY AND PHYSIOLOGY of ROTENOIDS IN SOME PAPILIONACEAE. I

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(With Plates XXXV–XXXVIII and 12 Text-figures)

CONTENTS

INTRODUCTION

PRELIMINARY work by Worsley & Nutman (1937) showed that histological methods can be used in studying the distribution of rotenone and allied substances in *Derris elliptica*. This paper reports its extension to members of the Dalbergieae and the Galegeae, the tribes that include genera of insecticidal value. I have therefore abandoned the former main heading of this series of papers, which was originally intended to be confined to *Derris* and *Mundulea*.

The species and plant parts listed in Table I have been available for study.

Table I. *Species and plant parts available for study*

Tribe	Genus	Species	Parts available for study
Galegeae	*Mundulea*	*sericea** Chev.	All parts
	Tephrosia	*Vogelii* Hook.f.	All parts
		candida DC.	All parts
		Ehrenbergiana Schweinf.	All parts
		toxicaria Pers.	All parts
		macropoda Harv.	Seeds and seedlings only
		densiflora Hook.f.	All parts except seeds
		rigida Bak.	Roots, stems, leaves
		purpurea Pers.	All parts
		congestiflora Harms.	Roots, stems, leaves
	Millettia	*dura* Dunn.	All parts
		Bussei Harms.	Seeds only
		Stuhlmanii Taub.	Seeds only
		usaramensis Taub.	Seeds only
Dalbergieae	*Derris*	*uliginosa†* Benth.	All parts
		elliptica Benth.	All parts except flowers and seedlings
		Malaccensis Prain	All parts except flowers and seedlings
		polystachya Benth.	All parts
		dalbergioides Bak.	All parts
	Lonchocarpus	*Bussei* Harms.	All parts except roots and flowers
		Capassa Rolfe.	Seeds only

* Although I am not satisfied that two species of *Mundulea* do not exist, I am using the specific name *sericea*, rather than *suberosa*, in conformity with present taxonomic practice.

† I have been informed that this African species is now known as *Derris trifoliata* Lour.

PROCEDURE AND TERMINOLOGY

Details of the application of Durham's test to histological work are given by Worsley & Nutman (1937). In brief, the method consists of treatment with nitric acid followed by ammonia solution of thick sections cut from fresh material with a moistened microtome knife: cells containing rotenone and some allied substances show a fugitive blue or green colour. Also, in certain tissues where such cells are morphologically distinct and can be recognized in sections cut from wax-embedded material, 6μ sections have been used in this study.

I have, hitherto, used the term "rotenone" to include all those naturally occurring substances which answer to Durham's test. This nomenclature is unsatisfactory, because confusion may arise between the actual substance rotenone and the group included in my extension of the meaning of the word, while with plants containing little or no rotenone, such as *Tephrosia Vogelii*, it is inappropriate. I shall, therefore, use the word ROTENOIDS,[1] which I define as those naturally occur-

[1] Purists might insist that rotenonoids is etymologically desirable: but I prefer rotenoids on the grounds of euphony and convenience.

ring substances which give a blue or green colour when treated with strong (about 75 %) nitric acid followed by strong ammonia solution.

For convenience, I shall refer to "rotenoid-cells" rather than to "rotenoid-containing cells", and to morphologically "distinct" rather than "specialized" cells. In the following descriptions all rotenoid cells are to be assumed to be not morphologically distinct unless otherwise stated.

PART I. THE DISTRIBUTION OF ROTENOIDS IN THE PLANT PARTS

I have studied all available parts of plants at all stages of maturity. Consequently, the presentation of the data in any systematic form is difficult and involves some repetition. A statement of general distribution appears in Table II, while a more detailed account follows.

Table II. *General distribution of rotenoids in the plant parts*

Name	Country of origin	Habit	Insecticidal value	Summary of tissues found to contain rotenoids
Mundulea sericea	Widespread in Africa	Tree	Moderate	Roots, stems, peduncles, ovaries, stamens, seeds
Tephrosia Vogelii	Africa—cultivated	Shrub	Moderate	Throughout the plant
T. Ehrenbergiana	Widespread in Africa	Herb	Unknown	Roots, stems, sepals, petals, ovules, stamens, seeds
T. candida	Tropical Asia	Shrub	Poor	Roots, stems, ovules, seeds
T. toxicaria	Tropical America	Shrub	Moderate	Roots, stems, sepals, petals, ovules, anthers, ovaries, seeds
T. macropoda	South Africa	Recumbent shrub	Moderate	Roots, stems, petioles, seeds
T. densiflora	West Africa	Shrub	Unknown	Roots, stems, petals
T. rigida	East Africa	Shrub	Unknown	None
T. purpurea	Tropical Africa	Herb	Slight	Roots, seeds
T. congestiflora	East Africa	Shrub	Unknown	None
Millettia dura	East Africa	Tree	Unknown	Roots, stems, seeds
M. Bussei	East Africa	Tree	Unknown	None
M. Stuhlmanii	East Africa	Tree	Unknown	None
M. usaramensis	East Africa	Tree	Unknown	Seeds
Derris uliginosa	General Tropics	Creeper	Unknown	Roots, stems, petioles, seeds
D. elliptica	Malay and Burma	Creeper	High	Roots, stems, seeds
D. malaccensis	Malay and Burma	Shrub	High	Roots
D. dalbergioides	Malay and Burma	Tree	Nil	Traces in seeds
D. polystachya	Himalayas	Shrub	Nil	None
Lonchocarpus Bussei	East Africa	Tree	Nil	None

A. Stems

Mundulea sericea.

Rotenoids can first be detected when the young plant is 5–6 weeks old, in the expanded ends of the medullary rays and occasionally in the cortex. Suberization is subsequent to rotenoid deposition, and I am satisfied that no causal relationship connects the two processes. Additional rotenoid cells later occur in the rays and the cortex, and afterwards in the pith. Finally, rotenoids can also be found in the secondary rays and in the phloem.

Mundulea stems become woody at a very early age, and rotenoids are then confined to the rays, the phloem and the cortex (Pl. XXXV, fig. 1), and occasionally in the medullary rays of the xylem: none is found in the almost completely lignified pith.

Because of the unavoidable thickness of the sections (necessary with Durham's test), it is sometimes difficult to identify the precise tissue that contains rotenoids: this is particularly so when sieve tubes are under examination. I have examined a very large number of sections of the phloem of *Mundulea*: rotenoids are often found in cells occurring among sieve tubes and similar in shape to them, and in at least two instances I have been able definitely to confirm their presence in a sieve tube with identifiable sieve-plates. I consider therefore that rotenoids occur in sieve tubes, and also possibly in companion cells.

A well-marked increase in rotenoids occurs at all nodes, beginning to be apparent when the leaf is developing and increasing until it is fully grown. In a young stem, 4 months old, the numbers of rotenoid cells found in three series of four $80\,\mu$ sections are shown in Table III.

Table III

	2–4 mm. below node				At the node				2–4 mm. above node			
	1	2	3	4	1	2	3	4	1	2	3	4
Pith	1	3	3	2	13	9	12	8	6	6	2	2
Phloem	2	2	2	2	3	3	1	3	1	3	1	3
Medullary ray and cortex	4	3	2	3	22	15	6	9	5	4	3	2
Total	7	8	7	7	38	27	19	20	12	13	6	7
Average		7				26				9·5		

Another similar stem gave averages of thirty-nine rotenoid cells at the node, twelve below it and fifteen above it. The increase is mostly in the pith, to a lesser extent in the medullary rays, and scarcely at all in the phloem. Later, when the xylem cylinder is well formed and most of the pith lignified, few rotenoid cells can be found in it: but the xylem is less lignified at the nodes, and increases in rotenoids occur here in the pith, the cortex and the rays. In mature trees the nodes on the main stems still contain more rotenoid cells than do the internodes. This increase is confined to the medullary rays and the cortex, since the pith is completely lignified and contains no rotenoids. As the pith in these nodes must originally have contained rotenoids the evidence for lability of these compounds is good, especially since they persist in dead tissues, and can be recognized therein.

Most of the rotenoid cells in *Mundulea* stems are above the average cell size for the tissues in which they occur: but since they are not larger than the maximum it is not generally possible to distinguish them in cleared sections. Rotenoids and starch have never been found in the same cell.

Tephrosia Vogelii.

The stems of this species become woody at a later stage than those of *Mundulea*, and the lignification of the pith is likewise postponed. The deposition of rotenoids is similar in most respects to that in *Mundulea*. They occur mainly in the medullary rays, but some also in the cortex and in the pith: those in the pith, unlike *Mundulea*,

tend to occur at its periphery. Because of the delayed lignification of the pith, rotenoid cells persist therein longer than in that of *Mundulea*.

Rotenoid cells are again concentrated at the nodes, and the main increase occurs in the pith.

None of the cells is morphologically distinct, and rotenoids and starch do not occur in the same cells.

Tephrosia Ehrenbergiana.

I have been unable to obtain any young plants of this species. Young stems of mature plants contain, in an $80\,\mu$ section, an average of one to two rotenoid cells, confined to the pith. Below the first branch, four or five cells occur in each similar section.

In this plant there is no doubt that rotenoids are first laid down in the pith; only in older stems can they be found in the cortex, where they tend to occur opposite the main medullary rays.

There is an appreciable increase in the number of rotenoid cells at the nodes, particularly in the cortex of main stems just below the point of branching: one cell was detected in a xylem medullary ray. In smaller stems, the internodes of which contain no rotenoids in the cortex, the increase at the nodes is confined to the pith, with only an occasional rotenoid cell in the cortex: a count showed an average of twelve cells per section in the internode and twenty-two at the node.

Tephrosia candida.

Only a very few rotenoid cells, confined to the medullary rays of the phloem and not morphologically distinct, could be found in the stems of this species.

Tephrosia toxicaria.

The larger stems contain a fair number of rotenoid cells in the pith and medullary rays of the phloem, and a few in the cortex. Small stems do not contain rotenoids.

Tephrosia densiflora.

A few rotenoid cells occur in the cortex and phloem of this species, frequently in the phloem rays. There does not appear to be any increase in the number at the nodes.

Derris elliptica.

Young stems of *D. elliptica* contain no rotenoids, but in an established plant, with one or more vines, many resin cells occur in the pith, and in the basal portion many of these also contain rotenoids. In a vine 360 cm. long, from a 2-year-old plant, rotenoid cells occurred in the lower 45 cm., the number gradually diminishing from the base up to this point. In vines 400 cm. long, from a 3-year-old plant, rotenoids were found up to 60 cm. from the base. They occur almost entirely in the pith, rarely in the phloem and cortex, and then only near the base of the stem.

In longitudinal sections the resin and rotenoid cells are often seen in chains: Pl. XXXV, fig. 2 illustrates this; nearly all the cells in the lower half of the photograph contain rotenoids, although the continuity is broken by occasional resin cells

containing no rotenoids. None of the cells is morphologically distinct; they can only be differentiated with certainty by Durham's test. The contents of the rotenoid cells are frequently more globular.

In the previous paper (Worsley & Nutman, 1937) it was stated that rotenoids could not be found in the cuttings that were used. While this was true for that material (which was all obtained from the upper, rotenoid free stems), it is not always so, for cuttings from the rotenoid-containing basal portions of *Derris* stems contain rotenoids which persist. This original rotenoid content remains if the cuttings die; or if the apical part dies, as sometimes happens, there is no evidence that the rotenoids are translocated from it.

As the plant grows, rotenoids are developed in the pith, whether any were already present there or not.

A woody cutting contained forty to fifty rotenoid cells per section, 80 μ thick, in the pith only: when it had developed a vine 110 cm. long it was found to contain seventy to eighty such cells per section, also only in the pith. A shoot also contained a fair number, and the base of the vine a few.

Subsequently, rotenoid cells can be found in the phloem, but only in the portions below soil level. Here, also, numerous resin cells occur in the medullary rays of the xylem, and occasionally one of these contains rotenoids.

An increase in the number of rotenoid cells at the nodes is rare.

Derris uliginosa.

The stems of this species grow to a great length, vines 7 m. long being not uncommon. Rotenoid cells occur in the pith in moderate quantity along almost the whole length of the stems. In stems about 7 m. long, sections 80 μ thick show about forty rotenoid cells per section in the first 50–100 cm. from the base. The number slowly diminishes up the stem; at 5 m. it is about eight to ten per section, at 6 m. four or five and at 6·5 m. only one or two; at 20 cm. from the top only an occasional cell can be detected. At the base of the stem a fair number of rotenoid cells also occur in the cortex, but the colour with Durham's test is very fugitive, which probably indicates a low rotenoid content in each cell. The cortical rotenoid cells also diminish in number up the stem, and cease at 2–2·5 m., except at the nodes. An occasional rotenoid cell can also be found in the phloem.

At the nodes of main branching there is an increase of rotenoids in the cortex: thus in one stem where they had ceased in the internodes at about 250 cm. up, they were present in moderate number around a main branching 350 cm. up.

The rotenoid cells are isolated and not in chains as with *D. elliptica.*

Millettia dura.

Woody stems of this species contain a few rotenoid cells in the cortex: none has been detected in either pith or phloem, nor in young stems.

B. *Roots*

Mundulea sericea.

Rotenoid deposition in the root is very similar to that in the stem; but rotenoids do not appear until after the completion of the xylem cylinder, and are rarely found in the pith. The medullary rays and cortex contain more rotenoid cells than do the

corresponding parts of the stem (Pl. XXXV, fig. 3). In young roots many rotenoid cells are filled with small globules (Pl. XXXV, fig. 4).

The number of rotenoid cells increases at the points of branching, this increase being entirely confined to the rays and the cortex.

Tephrosia Vogelii.

Rotenoid deposition in these roots is very similar to that in *Mundulea*. Rotenoid cells appear at a slightly earlier stage and more are found in the pith, these latter tending to be peripheral, as in the stems. An occasional rotenoid cell can be found in the xylem parenchyma, but most occur in the medullary rays, as may be seen in Pl. XXXV, fig. 5.

Tephrosia Ehrenbergiana.

Only a few rotenoid cells occur in these roots, mostly in the cortex, rarely in the phloem. In fine roots a few are found in the cortex, but not in any particular position, such as opposite the medullary rays. I have not found any in roots prior to suberization.

Tephrosia macropoda.

The roots of seedlings contain rotenoid cells in the cortex opposite the medullary rays, and a few in the rays. No larger roots were available for examination.

Tephrosia toxicaria.

In young roots rotenoids first occur in the cortex opposite the protoxylem and gradually spread to the xylem parenchyma and especially the medullary rays. Older roots contain appreciably more rotenoid cells than do other *Tephrosia* species.

Tephrosia candida.

A few rotenoid cells occur in the cortex and phloem: no particular arrangement of these cells, such as opposite the protoxylem, is apparent.

Tephrosia purpurea.

Rotenoids are first detected in the cortex opposite the protoxylem in young roots, thereafter spreading to the xylem parenchyma and around the cortex. Medium-sized roots contain appreciable quantities, but production at this stage slows down and large roots therefore contain a comparatively small proportion of rotenoid cells.

Tephrosia densiflora.

In young roots rotenoids are first detected in the cortex, approximately opposite the protoxylem. In older roots a moderate number of rotenoid cells occur in the cortex and phloem.

Derris elliptica.

For completeness, I repeat briefly the earlier findings on *Derris* roots.

Until suberization has begun no rotenoids can be detected. They first occur in the secondary cortex opposite the protoxylem and primary medullary rays when the plant is about 6 weeks old. Thereafter they gradually spread throughout the cortex

and xylem parenchyma, especially in the medullary rays. In a mature plant all the xylem parenchyma cells appear to contain either rotenoids or starch, but never both in the same cell.

Derris malaccensis.

Rotenoid formation in these roots resembles that in *D. elliptica*, and does not take place prior to suberization. Although not so definitely as in *D. elliptica*, rotenoids occur first in the cortex opposite the medullary rays, and gradually spread throughout the cortex and xylem parenchyma; but the number of the rotenoid cells is always appreciably less than in that species.

Derris uliginosa.

Initial deposition of rotenoids in the cortex opposite the protoxylem is very obvious in this species: all roots which I have examined have been tetrarch, and the second stage of rotenoid formation is in the cortex opposite the rays which form between the primary rays, i.e. at eight points, as illustrated in Text-fig. 1. Thereafter, rotenoid cells occur all around the cortex in moderate number; but only a few scattered ones appear in the medullary rays, even in large roots. This species therefore contains considerably fewer rotenoids in its roots than *D. elliptica* or *D. malaccensis*.

In young plants, rotenoids besides occurring opposite the protoxylem are found in the cortex around the points where rootlets branch off.

Millettia dura.

A few rotenoid cells occur in woody roots in the cortex only: non-woody roots do not contain any.

C. *Nodules*

Although leguminous these plants rarely produce nodules: possibly only when there is a nitrogen deficiency in the soil.

I have occasionally found a rotenoid cell in the cortex of a nodule from normally grown *Derris elliptica* and *Mundulea*. *Derris elliptica* plants grown in sand culture with a nitrogen deficiency produce large numbers of nodules, most of which contain a few rotenoid cells in the cortex and in the phloem.

D. *Petioles and leaves*

Tephrosia Vogelii.

Rotenoid cells occur in the petioles, usually in the pith, and occasionally in the phloem and cortex. No increase occurs where the petiolules branch off, and rotenoid cells are only rarely found in the latter. The rotenoid cells are above the average, but not appreciably above the maximum cell size of the tissue in which they occur.

The mid-ribs of the leaflets contain rotenoids in moderate amount: at the basal end of a mature leaflet about every other 80μ section contains at least one rotenoid cell, and some contain several (Pl. XXXV, fig. 6). The number of such cells diminishes along the mid-rib, and near the apex only about one section in six contains rotenoids. The vascular bundle of the mid-rib is surrounded by a sheath of sclerenchyma, often but sometimes not quite entire. Between the xylem and the adaxial sclerenchyma there are sometimes non-lignified cells about the size of the collenchymatous cells,

and frequently one of these contains rotenoids: usually however rotenoids occur only in the collenchyma.

Numerous, large, morphologically distinct rotenoid cells occur in the leaflet blades. They measure about $60 \times 50 \times 40\,\mu$ as compared with $20 \times 15 \times 15\,\mu$ for normal cells, i.e. 27 times as large, and lie with their longest side parallel with the mid-rib. They generally occur towards the adaxial side of the spongy mesophyll: Pl. XXXV, fig. 7, shows a transverse section through a mature leaflet.

The proportion of rotenoid cells increases with the maturity of the leaflet. Some are present from the earliest stages, and large, morphologically distinct rotenoid cells, about 20 times the volume of the surrounding ones (i.e. $30 \times 33 \times 37\,\mu$ as compared with $11 \times 11 \times 15\,\mu$), can be found in the plumule of an imbibed, but ungerminated,
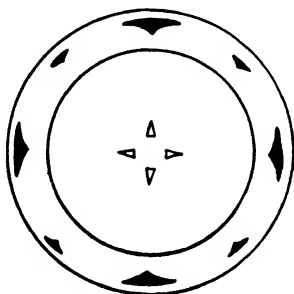
Text-fig. 1.   Text-fig. 2.

Text-fig. 1. Rotenoid deposition in *D. uliginosa* roots. ▷, protoxylem; ♥, groups of rotenoid cells in the cortex.
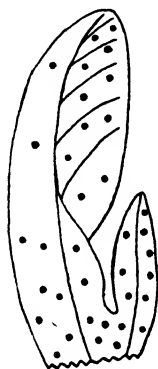
Text-fig. 2. Distribution of rotenoid cells (black) in plumule of *T. Vogelii*.

seed (Text-fig. 2). As the leaves unfold the proportion of rotenoid cells increases, and in a seedling with two leaves the younger contains about half the number in the older, which itself contains about its full complement. The petioles and petiolules of these young seedlings contain no rotenoids.

Derris uliginosa.

I have found a few rotenoid cells in the pith and phloem of the petioles, but none in the leaves.

Other species.

No rotenoids have been detected in either petioles or leaves of any other species.

E. *Peduncles*

Rotenoid cells are fairly numerous in both *Mundulea sericea* and *Tephrosia Vogelii* at the junctions of the peduncles with the stem and with the flower. In the former species a few such cells can usually be found along the length of the peduncle, always

in the pith: the pith also containing most of the cells that occur at the junctions. In *T. Vogelii* considerably more rotenoid cells occur: these are mostly in the phloem, but some are also in the pith. Although sometimes appearing slightly swollen these rotenoid cells are not morphologically distinct.

I have not detected rotenoids in the peduncles of any other species.

F. *Sepals and petals*

Tephrosia Vogelii.

From a very early stage the sepals and petals contain rotenoid cells, confined to the parenchyma and most numerous towards the base of the organ. These are morphologically distinct, and are illustrated in Pl. XXXV, fig. 8 and Text-fig. 3.
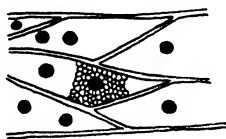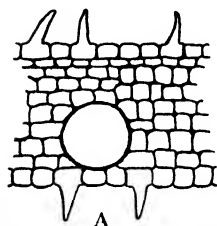
Text-fig. 3.

Text-fig. 4.

Text-fig. 3. Rotenoid cells in sepals and petals of *T. Vogelii*. *A*, sepal, transverse: showing one large rotenoid cell. *B*, petal, longitudinal: rotenoid cells black.

Text-fig. 4. Distribution of rotenoid cells (black) in *T. Ehrenbergiana* petals.

In the sepals they are very constant in size, about 19 times that of the surrounding cells ($70 \times 80 \times 50\,\mu$ as compared with $25 \times 30 \times 20\,\mu$). In the petals they are 3–4 times as numerous as in the sepals and of the same size: but since the surrounding cells are smaller than in the sepals ($22 \times 27 \times 17\,\mu$), they are about 27 times the size of their neighbours.

Even in extremely young sepals and petals from small buds the morphologically distinct cells almost invariably contain rotenoids: it is therefore probable that they are not differentiated much in advance of rotenoid deposition. It will subsequently be shown that this is the opposite to what occurs in stamens and ovules.

Morphologically distinct cells, identical with the rotenoid cells in appearance, occur in the sepals and petals, especially the latter. They contain an indicator pigment, colourless in untreated sections, turning magenta-red on addition of nitric acid, and becoming colourless again on further addition of ammonia: they never contain rotenoids. Similar cells occur in the ovary walls, in young green stems and occasionally in the bases of filaments.

Tephrosia Ehrenbergiana.

The petals and sepals of this species also contain morphologically distinct rotenoid cells, but unlike those in *T. Vogelii* they are long and narrow. In the petals their average size is $95 \times 30 \times 27\,\mu$ compared with normal cells varying between $30 \times 20 \times 25\,\mu$ and $45 \times 15 \times 15\,\mu$. They are more frequent near to the mid-rib, especially towards its basal end (Text-fig. 4). In one count they averaged thirteen per petal. In the sepals they are approximately the same size but are more scattered; they averaged twelve for a sepal twice the area of a petal.

Tephrosia densiflora.

A very few morphologically distinct rotenoid cells occur in the petals of this species: in twelve petals six such cells were found. They are about 21 times the volume of normal cells ($105 \times 52 \times 45\,\mu$ compared with $27 \times 22 \times 20\,\mu$). The sepals do not contain rotenoids.

Base   Middle
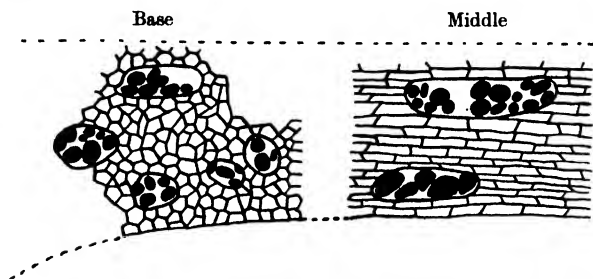
Text-fig. 5. Rotenoid cells containing rotenoid globules (black) in the filament of *Mundulea*: longitudinal.

Tephrosia toxicaria.

About one morphologically distinct rotenoid cell per sepal can be detected, $4\frac{1}{2}$ times the size of normal cells ($110 \times 25 \times 22\,\mu$ compared with $50 \times 18 \times 15\,\mu$). One or two per petal can also be found, $3\frac{1}{4}$ times normal size ($150 \times 22 \times 22\,\mu$ compared with $60 \times 18 \times 18\,\mu$); the contents are globular.

G. Stamens

Mundulea sericea.

The cortical tissues of the filaments contain rotenoid cells which are frequent at the base, sparse along the length and moderately frequent at the apex.

Since the rotenoid cells are morphologically distinct and many times the size of their neighbours, they can be studied in sections prepared from embedded material. It is sometimes possible so to embed the material that the contents of these large cells remain intact. Pl. XXXVI, figs. 1, 2 show such cells in transverse sections of the base and the centre of a filament, and Text-fig. 5 longitudinal sections of similar cells. The globular nature of the contents is apparent: it can also be seen in sections of fresh material, and here all the individual globules react to Durham's test, except in sections from very young buds, when some of the globules are found not to contain rotenoids.

The rotenoid cells along the filament are longer and narrower than those in the base. The former are about 5 times the size of their neighbours ($95 \times 45 \times 40 \mu$ compared with $55 \times 30 \times 20 \mu$). Those in the base are about 40 times the size of normal cells ($85 \times 90 \times 60 \mu$ compared with $25 \times 25 \times 17 \mu$). Those in the apex are similar in size and shape to those in the base.

Rotenoid cells also occur in the connective tissue of the anther, and are about 80 times the volume of the surrounding cells ($85 \times 65 \times 65 \mu$ as compared with $12 \times 15 \times 25 \mu$). Their contents are often globular, but not so noticeably as in the cells of the filament (Pl. XXXVI, fig. 3).

In very small flower buds a few morphologically distinct cells can be detected in the connective tissue of the anthers and in the base of the filaments: these cells are considerably larger than the surrounding ones and contain globules of resins or oils, but no rotenoids. In slightly larger buds some of these cells contain rotenoids, but only a few of the globules in each cell sometimes answer the test. As the buds increase in size more rotenoid cells can be detected, including a few along the filament stem, and all their globules contain rotenoids.

It is thus established that the rotenoid cells in the stamens are predestined to contain rotenoids. In my experience some sections have shown that not all the morphologically distinct cells contain rotenoids, but there is no reason to suppose that all may not contain them at a later date.

Rotenoids are first detected in the anthers about the time when the pollen-grains become differentiated: I have not been able to detect them in any anthers before this process, but I have found anthers, in which the pollen-grains are differentiated, without rotenoids. The two phenomena occur at the same time, but no evidence for or against any direct relationship between them has been discovered.

Tephrosia Vogelii.

Morphologically distinct rotenoid cells occur in both filaments and anthers of this species. They are more frequent but relatively smaller than in *Mundulea*. At the base of the filament they measure about $60 \times 40 \times 50 \mu$ as compared with normal cells of about $30 \times 15 \times 40 \mu$, i.e. 7 times the size: along the filament length they are about 3 times the size ($50 \times 35 \times 45 \mu$ compared with $15 \times 20 \times 85 \mu$).

In the anthers they are about 40 times as large ($80 \times 50 \times 60 \mu$ as compared with $20 \times 15 \times 20 \mu$ for normal cells). Here however they vary considerably, and the above figures are only very approximate, but on the average they are smaller than in *Mundulea*. Pl. XXXVI, fig. 4 shows a transverse section of an anther.

As with *Mundulea*, the rotenoid cells are differentiated before rotenoids are deposited in them; but the pollen-grains are frequently formed before rotenoids can be detected.

Tephrosia Ehrenbergiana.

The anthers contain a larger proportion of rotenoid cells in their connectives than any other species that I have examined: these cells often appear as a chain along both sides of the connective. They are morphologically distinct and measure about $45 \times 45 \times 45 \mu$, compared with normal cells of about $15 \times 12 \times 15 \mu$, i.e. they are 34 times as large.

In the filaments, rotenoid cells occur in moderate numbers all along their length: in one batch examined the average number was twelve per filament. Their size is about $67 \times 30 \times 37 \mu$, compared with about $90 \times 15 \times 22 \mu$, or $2\frac{1}{2}$ times: they are wider but somewhat shorter than normal cells.

Tephrosia toxicaria.

The anthers contain a large number of morphologically distinct rotenoid cells about 10 times the size of the surrounding cells ($65 \times 27 \times 27 \mu$ compared with $22 \times 15 \times 15 \mu$): they occur in the connective tissue only, and are longer compared with their width than similar cells in other species. I have been unable to detect any rotenoids in the filaments.

Tephrosia candida, Derris uliginosa and *Millettia dura.*

Neither anthers nor filaments contain rotenoids, but morphologically distinct resin cells occur, especially in the connectives.

H. *Carpels and seeds*

Mundulea sericea.

Unfertilized ovules of this species normally contain three rotenoid cells which are always in about the same position. Three can be seen in longitudinal sections (see Pl. XXXVI, fig. 5) in which only the three dark cells in the integument contain rotenoids. Only two can be seen in transverse sections (Pl. XXXVII, fig. 1). These cells are morphologically distinct, as may be seen in Pl. XXXVII, figs. 1–3. They are at first filled with small globules, as in fig. 2, which later coalesce to form large ones, as in fig. 3.

In ovules from unopened buds the rotenoid cells are about 150 times as large as their neighbours ($75 \times 60 \times 35 \mu$ compared with $15 \times 10 \times 7 \mu$). Their longest side is parallel to the axis of the ovule, and they are more numerous and larger near the embryo-sac than elsewhere.

As with the anthers, the rotenoid cells are differentiated before they contain rotenoids, which are first demonstrable at the time of differentiation of the embryo-sac. It is not likely that there is any causal relationship between these two phenomena, since although I have never found rotenoids before differentiation of the embryo-sac, the converse is not always true.

Fertilization as such does not appear to affect the rotenoid cells, but concurrently with the subsequent growth of the ovule they increase in number, at first between the original cells and eventually all around the integument (Pl. XXXVII, fig. 2). The rotenoid cells are at first confined to the tissues that will eventually become the testa.

They remain of approximately the same size throughout the development of the testa, and are confined to the parenchyma. When the integument is fully developed they measure about $65 \times 55 \times 40 \mu$, the largest seen being $80 \times 60 \times 60 \mu$. In the dried mature seed they measure only $60 \times 40 \times 25 \mu$, but return to their former size when swollen with water. The size of the neighbouring cells increases during the seed growth, and in a mature seed the rotenoid cells are only about 16 times the size of their neighbours.

Rotenoids do not occur in the cotyledons until they are definitely differentiated. Pl. XXXVII, fig. 4 is a section through an unripe but fairly mature seed: the

cotyledons although nearly fully developed contain no rotenoids. Pl. XXXVII, fig. 5 shows a mature seed with many rotenoid cells in the cotyledons. As the seed matures rotenoids are formed in the cotyledons, mainly in the peripheral palisade cells, many of which contain fixed oils. Rotenoids generally occur in an oil cell, although all oil cells do not contain rotenoids. In an unripe seed they are not distinguishable from the surrounding cells, but in a ripe seed they are generally somewhat larger than their neighbours, which often appear somewhat compressed: occasionally they are definitely larger than the average.

At the time rotenoids appear in the cotyledons they can also first be found in the radicle, but not in the plumule. They form a closely packed ring of cells in the peripheral cortex, and sometimes also around the central stele; rarely in the pith: Pl. XXXVII, fig. 5 illustrates a typical distribution. In transverse section, these cells appear as slightly swollen normal ones, but longitudinal sections show that they are morphologically distinct, being 2–3 times as long as their neighbours. The plumule does not develop rotenoid cells, but many occur in the cotyledons near the attachment.

The rotenone content of the ripe seed is 0·5–1 %: owing to the presence of about 14 % of fixed oils the determination is difficult. Fixed oils and proteins are the main food reserves: sugars and starch could not be detected.

Tephrosia Vogelii.

(a) *Ovules and seeds.* Rotenoid deposition follows closely that in *Mundulea*: morphologically distinct cells predestined to contain rotenoids can be detected in very small ovules, and rotenoids appear in them about the time of differentiation of the embryosac, although I have sometimes detected them earlier. Pl. XXXVII, fig. 6 shows large rotenoid cells in young ovules, and Pl. XXXVII, fig. 7 one such cell. The peculiar appearance of the contents is caused by shrinkage of the globules of rotenoid-containing resins. The relative sizes of the cells is well illustrated. Rotenoid cells average $80 \times 45 \times 75\,\mu$ compared with normal ones of about $30 \times 15 \times 18\,\mu$, i.e. they are 33 times as large.

As in *Mundulea*, rotenoids do not occur in the cotyledons until they are fully differentiated, when they also appear in the radicle, and in the

Text-fig. 6. *T. Vogelii*, seed testa. *a*, palisade cells; *b*, colomn cells; *c*, parenchyma; *d*, aleurone cells. Two large rotenoid cells shown black.

plumule, especially towards its tip. Rotenoid cells are more scattered in these cotyledons than in *Mundulea*: they are not generally distinct morphologically, although many are slightly swollen, and a few are definitely larger than normal cells. Longitudinal sections frequently show distal accumulations of rotenoid cells.

Rotenoid cells are more generally distributed in the cortex and pith of the radicle than they are in *Mundulea*: in transverse sections they appear slightly swollen, but in longitudinal sections most are about twice the length of normal cells. In the plumule the rotenoid cells are morphologically distinct, as has been described on p. 657.

In the testa the rotenoid cells occur entirely in the parenchyma (Text-fig. 6),

which can be peeled off in strips: on treating these strips with Durham's reagents the rotenoid cells are readily seen (Pl. XXXVII, fig. 8).

The funicle does not contain rotenoid cells, but there is an increase in number in the integument where it joins.

Fixed oils and proteins form the main food reserves: sugars and starch are not present.

(b) *Ovaries.* From its earliest stages the ovary contains a few morphologically distinct rotenoid cells, generally situated towards the inner side of the spongy tissue of its walls (Pl. XXXVIII, fig. 1). They measure about $45 \times 45 \times 50\,\mu$, a few up to $150\,\mu$ long, compared with about $17 \times 17 \times 22\,\mu$ for normal cells, i.e. they are 16 times as large. As in the petals of this species, a number of morphologically distinct cells occur in the outer tissues of the ovary, containing an indicator pigment which turns red with acid and then becomes colourless on addition of ammonia.

(c) *Styles and stigma.* A few morphologically distinct rotenoid cells, four times the size of their neighbours, occur along the style, in the cortical tissue. Pl. XXXVIII, fig. 2 shows eight such cells (round and black), near the outer edges of a transverse section. The stigma does not contain any.

Tephrosia Ehrenbergiana.

Young ovules of this species do not contain rotenoids, which can only be detected in the fully differentiated cotyledons, and in the radicle. In the ripe seed a fair number of rotenoid cells occur in the cotyledons, particularly at both ends: the palisade cells rarely contain rotenoids. Although similar in outline to their neighbours they are about three times larger (about $60 \times 50 \times 45\,\mu$ compared with about $45 \times 35 \times 30\,\mu$). In the radicle rotenoid cells occur in the outer layers of the cortex, and also around the central stele: viewed transversely they appear as somewhat swollen normal cells, but longitudinally many of them are twice the length of normal ones, as if produced by fusion of two cells.

As would be expected from the absence of rotenoids in the young ovule, the testa contains none: nor does the plumule.

Fixed oils and proteins form the chief food reserves.

Tephrosia candida.

A moderate number of rotenoid cells occur in young ovules, in the integument. As in *T. Vogelii*, rotenoids occur prior to differentiation of the embryo-sac, and have even been detected in the youngest ovules obtainable. The rotenoid cells are 33 times the size of their neighbours ($60 \times 37 \times 40\,\mu$ compared with $15 \times 12 \times 15\,\mu$).

As the cotyledons become differentiated rotenoids appear, in normally sized cells, particularly in the sub-epidermal layer. In the radicles, rotenoid cells are similar to those in *T. Ehrenbergiana*, appearing transversely as somewhat swollen normal cells, but longitudinally about twice the length of such cells (Text-fig. 7).

A few rotenoid cells occur in the parenchyma of the testa: they are only slightly larger than the surrounding cells, and are not filled with rotenoids but contain isolated globules of them (Text-fig. 8).

The plumule does not contain rotenoids. The main food reserves are fixed oils and proteins.

Tephrosia toxicaria.

(a) *Ovules and seeds.* A few morphologically distinct rotenoid cells can be detected in very small ovules from unopened buds: in ovules from open flowers a fair number occur, measuring about 43 times the size of normal cells ($37 \times 30 \times 22 \mu$ compared with $10 \times 8 \times 7 \mu$).

In imbibed mature seeds the testa contains rotenoid cells, in the parenchyma only, 24 times the size of the surrounding cells ($37 \times 22 \times 22 \mu$ compared with $15 \times 7 \times 7 \mu$).

Many large resin cells occur in the cotyledons and radicle: a fair number of slightly swollen normal cells containing rotenoids are also present. In the cotyledons the rotenoids are concentrated at each end, and in the radicle in the peripheral cortex especially at the abaxial side away from the cotyledons (Text-fig. 9).

Fixed oils and proteins are the chief food reserves.

Text-fig. 7.
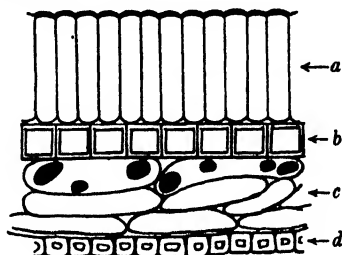
Text-fig. 8.

Text-fig. 7. Longitudinal section through part of *T. candida* radicle: to show relative sizes of rotenoid cells (black).

Text-fig. 8. Seed testa of *T. candida*. *a*, palisade cells; *b*, colomn cells; *c*, parenchyma; *d*, aleurone cells. Rotenoid globules shown black.

(b) *Ovaries.* A few morphologically distinct rotenoid cells are found in the spongy tissue of the ovary, usually at the junctions of the two walls. They measure about $3\frac{1}{2}$ times the size of the surrounding cells ($30 \times 22 \times 22 \mu$ compared with $18 \times 15 \times 15 \mu$).

(c) *Styles.* A few rotenoid cells occur near the base only of the style: they are 3 times the size of normal cells ($30 \times 22 \times 22 \mu$ compared with $37 \times 11 \times 11 \mu$).

Tephrosia macropoda.

The following description is based on the only two seeds available.

The testa contains a fair number of morphologically distinct rotenoid cells in the parenchyma, about 29 times as large as their neighbours ($110 \times 60 \times 35 \mu$ compared with $35 \times 15 \times 15 \mu$).

There are also a fair number of scattered normal sized rotenoid cells in the cotyledons, and the radicle contains rotenoids around its outer cortical layers, in cells frequently twice the length of normal ones, as if formed by fusion of two. The plumule is free from rotenoids.

Tephrosia purpurea.

Only small and moderate-sized ovules were available: in the former no rotenoids occur. The cotyledons contain some rotenoid cells, which appear to be normal ones somewhat swollen. None occurs in the testa.

Tephrosia densiflora.

Only small ovules from buds and flowers, and nearly ripe seeds were available. The former contain no rotenoids: the latter have a fair number of rotenoid cells in the testa, $10\frac{1}{2}$ times the size of normal cells ($65 \times 52 \times 45\,\mu$ compared with $45 \times 22 \times 15\,\mu$). Rotenoids are slow in appearing in the cotyledons of this species: the fully differentiated cotyledons contain many resin cells, but not until the seed is nearly ripe do any of these develop rotenoids: at this stage the radicle has none: the cells are $1\frac{1}{2}$ times normal size ($37 \times 30 \times 30\,\mu$ compared with $30 \times 30 \times 22\,\mu$).
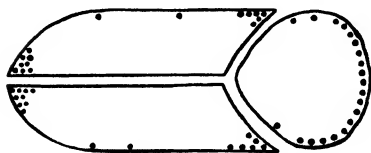
Text-fig. 9.
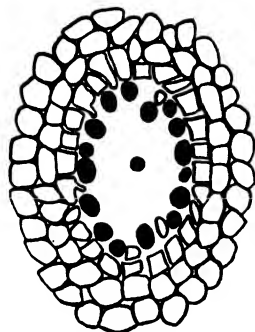
Text-fig. 10.

Text-fig. 9. Longitudinal section through ripe seed of *T. toxicaria*: to show distribution of rotenoid cells (black).

Text-fig. 10. Lysigenous cavity in *D. elliptica* seed: rotenoid globules black.

Derris elliptica.

This species has never flowered at Amani, but through the kindness of the Department of Agriculture of the Straits Settlements and Federated Malay States I obtained some seeds, but unfortunately none was viable. *Derris* seeds are very much larger than those of *Mundulea* or *Tephrosia Vogelii*, being $16 \times 9 \times 3$ mm. as compared with $5 \times 3 \times 1\frac{1}{2}$ and $6 \times 3\frac{1}{2} \times 2$ respectively. Their chief food reserve is starch, which occurs in transparent spherical grains, and not fixed oils. The cut seed, like the root, exudes a milky juice containing rotenoids and starch. Rotenoids occur in the cotyledons in large lysigenous cavities, formed by cell disintegration (Pl. XXXVIII, fig. 3 and Text-fig. 10). These are readily visible to the naked eye, averaging $300 \times 185 \times 135\,\mu$, the largest seen being $435 \times 230 \times 165\,\mu$, compared with normal cells of about $70 \times 45 \times 35\,\mu$, i.e. 68 times as large. Many of them are completely filled with rotenoid containing resins, but some only contain a globular coating around their walls. Occasionally an empty cavity was seen, but this may have been due to loss of its contents during sectioning. The radicles contain no rotenoids or cavities, but rotenoid cavities occur near the abaxial surfaces of the immature leaves of the plumule, averaging 76 times the size of the surrounding cells ($120 \times 70 \times 90\,\mu$ compared with $22 \times 15 \times 30\,\mu$). The available material was too shrivelled to photograph, but the appearance was very similar to Pl. XXXVIII, fig. 4.

Derris uliginosa.

I have only been able to obtain small ovules and half-ripe to fully ripe seeds: there is therefore a stage in the ovule growth which has not been examined.

In the small ovules no rotenoids can be detected, but a few morphologically distinct resin cells occur. In half-ripe seeds the cotyledons, which are already differentiated, contain large lysigenous cavities of rotenoids, very similar to those in *D. elliptica*: owing to the lack of material I am unable to say whether these rotenoids are formed before or after differentiation of the cotyledons, but on the analogy of other species it is probably afterwards. In addition to the many large cavities there are also a number of smaller ones. The large ones are 43 times the size of normal cells $(215 \times 145 \times 105 \mu$ compared with $45 \times 45 \times 37 \mu)$.

The plumule contains similar cavities, 43 times the size of normal cells $(240 \times 100 \times 90 \mu$ compared with $45 \times 37 \times 30 \mu)$: these occur near the abaxial surface of the immature leaves, and near the tip (Pl. XXXVIII, fig. 4).

The radicle does not contain rotenoids, but large rotenoid cavities generally occur in the cotyledons just below the junction; one measured $410 \times 150 \mu$ in longitudinal section.

No rotenoids occur in the testa of the seed. Starch, and not fixed oils, is again the food reserve.

Derris dalbergioides.

Seeds of this species contain large cavities filled with resins in their cotyledons. A very faint and transient blue colour with Durham's test is sometimes obtained in a few of the cavities, indicating the presence of traces of rotenoids. No other parts of the seed answer to the test. The food reserve is starch.

Millettia dura.

Although this tree flowers prolifically it appears to set very few seeds: I have only been able to obtain small ovules from buds and flowers and a few ripe seeds, no intermediate stages being available.

Small ovules do not contain rotenoids. The seeds are large, about $12 \times 10 \times 2$ mm., and on removing the testa, which is free from rotenoids, long rotenoid cavities near the surface are plainly visible to the naked eye (Pl. XXXVIII, fig. 5). Sections show many large lysigenous cavities similar to those in *Derris* species, and apparently all of them contain rotenoids. The cavities average 27 times the size of surrounding cells $(200 \times 105 \times 105 \mu$ compared with $60 \times 45 \times 30 \mu)$: some however are very much larger, and one of those visible in Pl. XXXVIII, fig. 5 measured $1575 \times 340 \times 250 \mu$, or 1650 times the surrounding cell size.

The radicle also contains similar cavities of rotenoids mostly in the outer cortex: they are about 10 times normal cell size $(180 \times 160 \times 150 \mu$ compared with $52 \times 37 \times 22 \mu$: the largest seen measured $240 \times 180 \times 150 \mu)$.

The plumule contains rotenoid cavities immediately below the abaxial surface of the immature leaves: see Pl. XXXVIII, fig. 6, in which the cavities can be seen to have caused a localized expansion of the epidermis. The cavities are about 38 times the size of normal cells $(80 \times 50 \times 47 \mu$ compared with $10 \times 10 \times 7 \mu)$.

Starch, and not oils, forms the main food reserve.

Millettia Bussei and *M. Stuhlmanii.*

The cotyledons of both these species, especially the former, contain large lysigenous cavities, but no rotenoids. Starch is the food reserve in both.

Millettia usaramensis.

Only a few old and shrivelled seeds of this species were available. Rotenoids were present in large cavities in the cotyledons and radicles: in the former they measured about 87 times the size of normal cells ($200 \times 105 \times 90\,\mu$ compared with $45 \times 22 \times 22\,\mu$). The radicle was too shrivelled for measurements to be made. Traces of rotenoids were apparent in the very shrivelled plumules.

PART II. GERMINATION PHENOMENA

The most interesting and important fact elicited during this investigation is the behaviour of rotenoids during germination. Unfortunately, no viable seed of *Derris elliptica* has yet been obtained.

Mundulea sericea.

On germination the testa splits and is discarded, its rotenoids remaining in it undiminished. As the radicle elongates, additional rotenoid cells are formed throughout its length to the growing point. Those near the tip are generally 2–3 times the length of normal cells: they often appear as if formed by two cells coalescing (Text-fig. 11). Occasionally they are also double the width. In the basal two-thirds the rotenoid cells are much longer than normal ones (Text-fig. 11), and they form almost a network around the outer cortex. They vary between 3 and 8 times the size of normal cells ($75 \times 20 \times 20\,\mu$ to $150 \times 30 \times 20\,\mu$ compared with $30 \times 22 \times 17\,\mu$; one $350 \times 40 \times 20\,\mu$ was seen).

Later, as the radicle further increases in length, the rotenoids begin to disappear, from the tip first: when it is about 10 mm. long a definite reduction is noticeable; when about 15 mm. only a few rotenoid cells remain in the apical half, although many still remain in the basal half. After suberization has begun, usually when the radicle is about 20 mm. long, no rotenoid cells can be found except near the base, and these are greatly reduced in number. At this stage, rotenoids can be found only in the basal part of the cotyledons, since they have at the same time been slowly disappearing, starting at the distal ends. When the radicle is about 40 mm. long, only a few rotenoid cells can be found, at the junction of the cotyledons and the radicle: soon afterwards none can be detected anywhere in the seedling (Text-fig. 12).

There is thus a definite movement of rotenoids, first towards the newly growing tissue of the radicle, and secondly throughout the entire seedling, with their eventual complete disappearance.

Until the seedling is about six weeks old no further rotenoids can be detected in it.
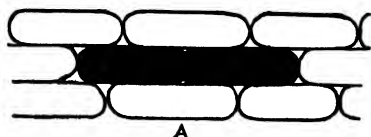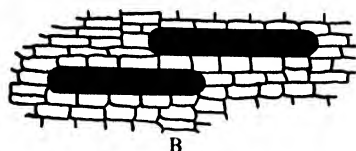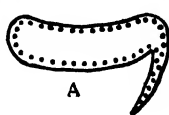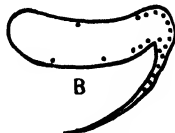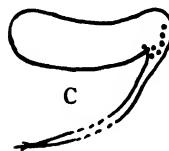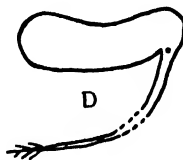
Text-fig. 11.   Text-fig. 12.

Text-fig. 11. Rotenoid cells (black) in the growing radicle of *Mundulea: A*, near the tip; *B*, in the centre. Transverse.

Text-fig. 12. Distribution of rotenoid cells (black) in a germinating *Mundulea* seed. *A*, shortly after germination; *B*, radicle, 20 mm. long; *C*, radicle, 40 mm. long; *D*, radicle, 60 mm. long.

Tephrosia Vogelii.

In general, seeds of this species behave in the same way as those of *Mundulea* on germination.

The testa is discarded without apparent loss of its rotenoids: the cotyledons lose most, but not all, of theirs. During growth of the radicle rotenoid cells increase in number and occur throughout its length, most of them being somewhat larger than normal, but seldom as large as in *Mundulea*; later the number decreases, and when the radicle is about 20 mm. long its tip has lost most of its rotenoids. When it is about 60 mm. long, a few can be found at its base only: when it is larger none can be detected. The cotyledons meanwhile lose most, but not all, of their rotenoids: thus in a seedling 10 cm. long, with several rootlets, $80\,\mu$ longitudinal sections showed an average of twenty-two rotenoid cells per section, compared with 114 in ripe seeds. When the cotyledons wither, rotenoid cells can still be detected in them.

Rotenoids exist in the plumule in morphologically distinct cells, as

already described on p. 657; these increase in number on the opening of the plumule: no loss has ever been observed.

The fact that rotenoids are found in the newly grown tissue means that either new supplies of them have been produced, or that they have been translocated from the cotyledons. As viable seeds of this species were plentiful, experiments were carried out to settle this point.

The radicles were dissected from 200 ripe seeds, and from 200 germinated ones with radicles about 10 mm. long, and the rotenoid contents of these two samples and of the companion cotyledons were determined colorimetrically by the methods of Gross & Smith (1934), and of Roger & Calamari (1936). In addition, in one determination, 200 seedlings with fully open cotyledons were taken as a fifth sample.

Percentage results cannot be used because of changes in weight, moisture content, etc., during growth; and therefore the weight of rotenoids per 100 seeds or seedings has been calculated for each sample. The two colorimetric methods gave different absolute results, but the ratios between them were constant, and the percentage increases of rotenoids obtained between the samples can therefore be accepted; see Table IV.

Table IV

	Ripe seed		Germinated seed		Fully open cotyledons	
	A mg.	B mg.	A mg.	B mg.	A mg.	B
Radicles	10·0	7·5	11·4	9·4	—	—
Cotyledons	15·0	10·1	18·8	11·7	—	—
Total	25·0	17·6	30·2	21·1	11·3	—
Ratio A/B	1·42		1·43		—	

Increase on germination: 20·8 % by A, 19·9 % by B; mean = 20·3 %.

The figures under columns A are those obtained by the method of Gross & Smith, and those under B by the method of Roger & Calamari. The results show (1) an increase soon after germination of about 20 % in the total amount of rotenoids, made up of increases in both the radicles and the cotyledons, and (2) a reduction, as growth of the seedling progresses, to a final amount of about 45% of the original in the ripe seed, or only 37·4 % of that in the newly germinated seedling. This experiment, therefore, establishes that rotenoids are actually produced in the young germinating seed, and that shortly afterwards a large proportion of the rotenoids disappears.

In a somewhat similar experiment with *Mundulea sericea*, in which, owing to shortage of material, the radicles were not separated from the cotyledons, a total increase in rotenoids of 14·8 % was obtained after germination, when the radicles were 5–10 mm. long.

Tephrosia Ehrenbergiana.

Germination phenomena in these species resemble those in *T. Vogelii*. At first, new cortical rotenoid cells are produced in the new tissue of the radicle, and can be detected there until it is about 7 mm. long (these seeds are much smaller than those of *T. Vogelii*). Thereafter, they begin to disappear from the tip upwards: when the radicle is about 15 mm. long none can be found in it. Rotenoids also diminish steadily in the cotyledons, and when these are fully open only a few rotenoid cells can be found in them: 80 μ sections of one pair of such cotyledons contained an average of thirteen rotenoid cells, against sixty-seven in a ripe seed. Even when they wither the cotyledons still have a few left in them.

Tephrosia candida and T. toxicaria.

These species behave very similarly to the above, except that the fully open cotyledons contain only a very few rotenoid cells: in several *T. candida* cotyledons the average was one in every four 80 μ sections, and in *T. toxicaria* it was slightly higher.

Tephrosia macropoda.

Only four seeds were available for germination tests, and the results are therefore meagre: they indicate that new rotenoid cells occur at first all along the growing radicle, as with the other species, and later disappear from the whole seedling except for a few in the cotyledons.

Derris uliginosa.

Germination phenomena in this species are different from those in the genera *Mundulea* and *Tephrosia*. The radicle in the seed does not contain rotenoids and the cotyledons contain them in large cavities and not in cells. On germination no rotenoids are produced in the radicle at any stage of its growth, and no new rotenoids appear to be formed in the cotyledons. As growth proceeds a small reduction of the rotenoid content of the cotyledons occurs: the original amount being reduced by 38 %, as determined by colorimetric methods. The rotenoids in the plumule disappear soon after germination and none can be detected when the plumule emerges from the cotyledons.

Millettia dura.

The cotyledons, radicle and plumule of this species all contain cavities of rotenoids. On germination new rotenoid cavities are formed along the radicle, down to the growing point: they occur almost entirely in the peripheral cortex. The cavities are large enough to be readily seen along the surface of the radicle by the naked eye: they are about 350 times the size of normal surrounding cells ($270 \times 90 \times 80\mu$ compared with $37 \times 10 \times 15\mu$). As growth proceeds the rotenoids in the radicle begin to diminish from the basal end first and by the time that it is about 10 cm. long no rotenoids can be found in the upper half, the structure of which is stem, but some rotenoids still occur in the lower or root half. At this stage long cavities can be found in the cortex of the radicle: those in the upper half contain resins only, whilst some of those in the lower half also contain rotenoids. Later, no rotenoids can be detected in the root. This process is, therefore, the opposite to what occurs in *Mundulea* and *Tephrosia* spp., where rotenoids commence to diminish at the tip. The cotyledons lose rotenoids from most of the cavities around their edges but little loss occurs in the body of them: there were insufficient seeds available for quantitative work, but I doubt if the reduction in total rotenoids is large.

The rotenoids in the plumule have disappeared by the time that it emerges from the cotyledons.

Summarized table of distributions

The preceding results are summarized in Table V, which shows that rotenoids occur in many different organs: in some species, viz. *Tephrosia purpurea*, in only a few, but in others, viz. *T. Vogelii*, in many tissues. The rotenoid cells in all the stems and roots are normal ones, but in most other tissues they are morphologically distinct.

It must be understood that the volume figures are only approximate, and are based on the average of a number of measurements: in some tissues the morphologically distinct cells vary greatly in size. In the seeds of *Derris elliptica*, *D. uliginosa* and *Millettia dura* the figures are for the lysigenous cavities compared with normal cells.

PART III. EXPERIMENTAL

The results recorded in Part I of this paper have been entirely observational, but they have suggested many provisional hypotheses to be tested by experiment. I have carried out some preliminary work of this nature and the results are recorded below.

Table V. Summary of plant parts containing rotenoids

Tephrosia

	Mundulea Sericea			Vogelii			Ehrenbergiana			candida			toxicaria			macropoda			densiflora			purpurea		
	Rote-noids	Type	Vol.	Rote-noids	Type	Vol.	Rote-noids	Type	Vol.	Rote-noids	Type	Vol.	Rote-noids	Type	Vol.	Rote-noids	Type	Vol.	Rote-noids	Type	Vol.	Rote-noids	Type	Vol.
Stems	+	N	1	+	N	1	+	N	1	+	N	1	+	N	1	+	N	1	+	N	1	+	N	1
Roots	+	N	1	+	N	1	+	N	1	+			+	N	1	+	N	1	+	N	1	+		
Petioles	−			+	N		+	N		−			−			+	N		−			−		
Leaflets	−			+	D	27	−			−			−			+			−			−		
Petiolules	−			+	N	1	−			−			−			−			−			−		
Peduncles	+	N	1	+	N	1	−			−			−			−			−			−		
Sepals	−			+	D	19	+	D	6	−			+	D	3½	+	D		+	D	21	−		
Petals	−			+	D	27	+	D	6	−			+	D	4	+			−			−		
Filaments, base	+	D	40	+	D	7	+	D	4	−			+	D	10	+			−			−		
stem	+	D	80	+	D	3	+	D	2¼	−			+	D	3½	+			−			−		
Anthers	−			+	D	40	+	D	34	−			+	D	43	+			−			−		
Ovaries	−			+	D	16	−			+	D	33	+	NS	1½	+	D	29	+	D	10½	+	NS	1¼
Styles	−			+	D	4	−			+	NS	1½	+	NS	1½	+	D	1	+	D	1½			
Ovules	+	D	150	+	D	33	+	D	3	+	N	1	−			−	D	2						
Seeds: testa	+	NS	16	+	D	9	+	D	2¼	+	N	2½												
cotyledons	+	D	1½	+	NS	1½																		
radicle	+	D	2½	+	D	2																		
plumule	−			+	D	20																		

Derris

	uliginosa			elliptica			malaccensis			dalbergioides			*Millettia* dura			usaramensis		
	Rote-noids	Type	Vol.	Rote-noids	Type	Vol.	Rote-noids	Type	Vol.	Rote-noids	Type	Vol.	Rote-noids	Type	Vol.	Rote-noids	Type	Vol.
Stems	+	N	1	−	N	1	+	N	1	−			+	N	1	+		
Roots	+	N	1	+	N	1	+	N		−			+	N	1	+		
Petioles	+	N	1	+			−			−			+			+		
Leaflets	−			−			−			−			−			−		
Petiolules	−			−			−			−			−			−		
Peduncles	−			−			−			−			−			−		
Sepals	−			−			−			−			−			−		
Petals	−			−			−			−			−			−		
Filaments, base	−			−			−			−			−			−		
stem	−			−			−			−			−			−		
Anthers	−			−			−			−			−			−		
Ovaries	−			−			−			−			−			−		
Styles	−			−			−			−			−			−		
Ovules	−			−			−			−			−			−		
Seeds: testa	+	D	43	−	D	68				−			+	D	27	−	D	
cotyledons	−									+	D		+	D	10	+	D	
radicle	+	D	43	+	D	76				−			+	D	38	+	D	87
plumule	−												−			−		

+ = rotenoids present; − = rotenoids absent; blank = tissue not available for examination; N = normal; NS = normal but somewhat swollen; D = morphologically distinct. The columns headed "Volume" give the average ratio of rotenoid cell volume to that of the surrounding cells.

A. Nutrition deficiency experiment

In order to determine the effect, if any, of various mineral deficiencies on the production of rotenoids in *Derris elliptica*, I set up a pot experiment with three replicates of clonal material in each treatment. Ten-inch flower pots were used, and the plants were grown in pure sand (obtained locally and consisting of over 99 % silica), and nutrient solutions applied regularly. Five treatments were given, viz. (1) Hoagland's full nutrient (see Eaton, 1936), (2) complete potash deficiency, (3) 95 % deficiency of nitrogen, (4) complete calcium deficiency and (5) complete phosphorus deficiency. Tap water (which in Amani is remarkably pure and contains only 12–15 p.p.m. of dissolved salts, most of which are silica) and ordinary pure chemicals were used and, therefore, the potassium, phosphorus and calcium deficiencies, although not complete, were very nearly so.

After 18 months the three roots in each treatment, regarded as one sample, were lifted, weighed, examined microscopically and analysed. Table VI shows the results.

Table VI

	Root dry wt. g.	Ether extract dry wt. %	Rotenone dry wt. %	Rotenone per plant g.
Full nutrient	255	6·0	1·55	1·3
K deficient	215	9·4	2·55	1·8
N deficient	240	10·9	3·05	1·6
Ca deficient	120	9·6	2·60	1·0
P deficient	54	8·7	2·45	0·4

They indicate that complete deficiencies in potash and nitrogen have little effect on the yield of root, whereas calcium deficiency reduces it to half and phosphorus deficiency to nearly one-fifth. The figures for the rotenone contents of the roots are surprising: they show that full nutrient solution gave only about half as much as that given by each of the four deficiency solutions. If worked out on a basis of weight of rotenone per plant, potash and nitrogen deficiencies, especially the former, show a greater production of rotenone than full nutrient.

The experiment will be repeated on a larger scale, but the present results suggest, *inter alia*, that a poor soil may be preferable to a rich one for high rotenone content.

One interesting point was that the plants grown with a deficiency of nitrogen produced large numbers of nodules on their roots, and that many of these nodules contained rotenoid cells. *Derris* plants grown

normally have few if any nodules, and I have seldom detected rotenoids in those that do occur.

As determined by microscopic examination, the distribution of rotenoids was normal in roots of all the plants except those grown with a calcium deficiency. In these latter roots few rotenoid cells occurred in the outer xylem parenchyma which showed as a ring of non-rotenoid cells (Pl. XXXVIII, fig. 7): small roots were, however, normal. All the roots contained many starch cells.

In what was the original cutting the distribution was normal for full nutrient and potash and nitrogen deficiencies: with calcium deficiency, there were scarcely any rotenoids in the part of the cutting above ground, whilst with phosphorus deficiency there were few in the part below ground: other distributions were normal.

On the whole, it can be said that none of the deficiencies produced any great change in the rotenoid distribution in the entire plant.

B. *Light deficiency experiment*

In order to determine whether the rotenoids in *Derris* root could be used as a food reserve, a number of potted plants were placed in the dark, watered regularly, and kept there until dead. Before dying, several of them produced long etiolated shoots.

Examination under the microscope showed the rotenoids apparently undiminished in quantity, although all the starch and much of the hemicelluloses had been used up. To test this quantitatively a plot of ten 1-year-old plants in the field was covered so that light was excluded: ten plants adjacent to them in the same plot being used as controls. When dead the former were lifted, weighed and analysed. Table VII shows the results, which indicate that if any loss of rotenoids has occurred it is only a small one.

Table VII

	Dry wt. of roots g.	Rotenone %	Rotenone per plant g.
Normal plants	400	7·4	3·0
Plants kept in dark	280	8·9	2·5

As the roots of those plants kept in the dark were more breakable, a certain amount was lost in digging, and if allowance be made for this loss the value for rotenone per plant is greater than 2·5 g. It is, therefore, very improbable that the rotenoids are available as a food reserve.

C. *Experiment with* Derris *cuttings*

Cuttings from the lower, woody parts of *Derris elliptica* stems root more readily than those from the upper, green parts: the former contain rotenoids in their pith, the latter do not.

In order to test if any connexion exists between these two facts, I took green cuttings, tested them at each end to ensure that no rotenoids were present, and then provided them with rotenoids in the form of (*a*) expressed *Derris* root sap, (*b*) expressed stem sap. These saps were applied (1) by hypodermic syringe into the pith at both ends, and (2) by dipping the lower end into the sap and applying suction to the top.

Ten cuttings were used in each test. A small reduction occurred in the percentage number of cuttings which rooted and no increase in the speed of rooting was noticeable.

With root sap added, 30 % of the controls rooted, while only 20 % with each treatment did so: at the same time 80 % of woody cuttings rooted. With stem sap, the corresponding figures were 60, 50, 50 and 80 %.

It appears, therefore, that the rotenoids in the lower end of *Derris* stems have no effect on their rooting qualities.

DISCUSSION

This exploratory study has provided information on rotenoid distribution in several species of plants, and has suggested lines for future investigation. Although some of the facts relate to certain species only, a number of generalizations appear to be justifiable.

In every species that contains rotenoids in any of its tissues there are rotenoids in the seeds and, with one exception, in the roots: the exception is *Derris dalbergioides*, the seeds of which contain such small traces of rotenoids that smaller traces in the roots may well have been overlooked altogether.

The species in which rotenoids have been detected show a range of rotenoid content from the mere traces in the seeds of *D. dalbergioides* to the appreciable quantities in almost every organ of *Tephrosia Vogelii*.

In six species grown from seed, rotenoids were first detected in the young roots, in the cortex opposite the protoxylem and primary medullary rays; thereafter spreading, to a greater or lesser extent, around the cortex and through the xylem parenchyma. In young roots of older plants the process is similar. There must be some significance in the fact

that rotenoids are so frequently laid down in the cortex opposite the protoxylem, but I am unable to suggest what it may be. The suggestion that it is only a question of waste products moving along the rays into what will eventually be the bark cannot hold, because the rotenoids soon spread to other tissues.

Of the species examined fully, four have rotenoids in their stamens, and four (not all the same four) in their ovules, all in morphologically distinct cells. Rotenoid cells in young ovules have, in every instance, persisted in the testa of the ripe seed. This appears to be the rule, so that, for example, with the knowledge that the testa of *T. macropoda* contains rotenoids, I confidently expect to find them in young ovules when flower buds become available.

I consider it possible that some chemical relationship exists between the rotenoids and the resins in these plants, for they generally occur together in the same cells. This is particularly true for mature *Derris elliptica* roots, where all resin cells in the xylem parenchyma contain rotenoids. In very young anthers and ovules of *Mundulea sericea* the resin cells contain no rotenoids, but, as the organs grow, first one or two and then gradually all of the resin globules in a cell are found to contain rotenoids. This indicates that the resins and rotenoids are intimately mixed in each cell. The pith of *Derris elliptica* stems contains many resin cells, and in the lower woody parts many also contain rotenoids: as the stem grows and the woody tissue extends upwards, more of the resin cells become rotenoid-containing. In *D. uliginosa* many of the resin cells all the way up contain rotenoids, irrespective of the woodiness of the stem. In some species the anthers and certain other tissues do not contain rotenoids, but resin cells are found that are similar in appearance to, and in the same positions as, rotenoid cells in other species.

In considering the relationship between the resins and the rotenoids it may be recalled that many resins on melting with potash yield resorcinol, and that an alkyl resorcinol has recently been obtained from the tubaic acid fraction of rotenone.

As far as I am aware no suggestions have been put forward by other workers as to the physiological role that rotenoids may play in plants. Two hypotheses at once present themselves, (1) that rotenoids play an active part in plant metabolism, e.g. as food reserves; (2) that they are waste products. My results so far suggest a third hypothesis, namely, that rotenoids play an active part in the germinating seed but are waste products in all other tissues.

On germination, *Mundulea sericea* and the five *Tephrosia* species of

which viable seeds were available behave very similarly: rotenoids increase in both radicle and cotyledons and then diminish, in the former to nil, in the latter to a varying degree, the extremes being by 55% in *T. Vogelii* and by 100 % in *Mundulea sericea*.

In *Millettia dura* the process is the same except for a smaller reduction in the cotyledons. In *Derris uliginosa* no initial increase occurs and the final reduction in the cotyledons is by 38 %. The rotenoids in the plumules of these two species, however, disappear entirely. This disappearance of rotenoids during germination is consistent with the third hypothesis.

In all other tissues, including the testa, rotenoids appear to be waste products, in the sense that they are not available as food reserves, since all these tissues can wither and die without any apparent loss of rotenoids, and this is so whether the rotenoid cells are normal or morphologically distinct.

Amani strain *D. elliptica* has been grown under varying conditions of rainfall, altitude, soil, etc., i.e. with about 100 in. of rain in the hills at 3000 ft. altitude, with about 50 in. at a few hundred feet elevation, under irrigation on the plains, and in sandy as well as very heavy soil. In every case the percentage rotenone content increased steadily, to a maximum, with the growth of the root.

This indicates that rotenone production in the root is a regular and constant process, as would be expected if rotenoids were waste products steadily accumulating during plant growth. Experiments have shown that the rotenoids are not reduced in amount by conditions that reduce food reserves, and this favours the hypothesis that they are waste products, not utilizable as food.

The increase in the number of rotenoid cells, especially in the pith, at nodes and to a lesser extent at root branchings, suggests that rotenoids may here be a by-product of secondary or lateral growth. Similar accumulations of rotenoids also often occur at other junctions, e.g. of the peduncle with the stem and with the flower, of the filament with the anther and with the receptacle, and of the funicle with the integument. I have also found large amounts of peroxidases at many of these junctions, especially at the nodes. Other workers have shown that accumulations of other substances, e.g. iron, occur at nodes. Nodes are in general points of active growth, and the presence of increased amounts of rotenoids here lends support to the idea that they are in some way directly connected with the growth of the plant; but as they remain undiminished after secondary or lateral growth has ceased, their presence cannot be

accounted for by the assumption that they are substances of use to the plant as a food supply.

Except in germinating seeds, only two instances are known of proven lability of rotenoids, i.e. their disappearance from the pith of *Tephrosia Vogelii* and *Mundulea sericea*, especially at the nodes. Rotenoids occur in the non-lignified pith but cannot be detected after lignification. I have no evidence to show whether these rotenoids have been translocated prior to lignification or destroyed during that process.

On the other hand I have detected rotenoids in tissues usually connected with translocation, e.g. sieve tubes and medullary rays: I am of the opinion, however, that rotenoids are not normally translocated as such. For instance, *Derris elliptica* plants grown from cuttings with no original rotenoids develop them only in the roots until the plants are fairly mature, and they must therefore have passed down the stem in some non-rotenoidal form and been converted to rotenoids in the root.

With *D. elliptica*, the root as it grows steadily accumulates rotenoids and starch until all the xylem parenchyma cells contain either the one or the other: except for their contents these cells appear identical. It is, therefore, probable that both starch and some form of rotenoid precursor are passing down continuously from the leaves to the root, where in some cells starch is stored and in others the rotenoid precursors, which are then converted to rotenoids *in situ*. Here the rotenoids appear to be waste products and, therefore, it may be assumed that the precursors have performed some useful function before being converted to waste products. It is also conceivable that simple substances may pass down from the leaves into the roots or other tissues, and then undergo a complex synthesis *in situ*: this, I consider, less likely.

It is very difficult to suggest in what way the rotenoids can be utilized in the plants. Rotenone, $C_{23}H_{22}O_6$, which is usually the chief toxic constituent to be isolated from these plants, has been assigned the structural formula
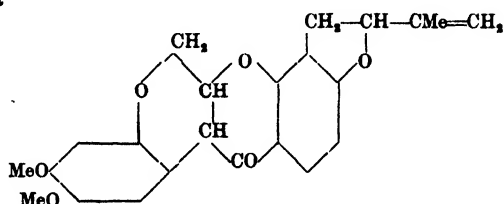
and chemically the other toxic constituents, tephrosin, deguelin and toxicarol, are closely allied: the first three all give derric acid, $C_{12}H_{14}O_7$,

on oxidation. Rotenone is considered to consist of a rissic acid fraction, $C_{11}H_{12}O_3$, containing two methoxy groups, combined by a carbonyl group to a tubaic acid fraction, $C_{11}H_{10}O_2$, thus $C_9H_6O(OCH_3)_2 . CO . C_{11}H_{10}O_2$. On oxidation *in vitro* several of the rotenoids yield rissic acid, $C_{11}H_{12}O_7$, with the structure

O.CH₂COOH

COOH

CH₃O

CH₃O
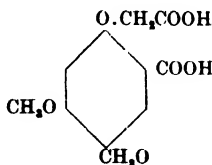
Whilst it is more likely that these less complex substances could be utilized by the plant, it appears unlikely that such complex substances as rotenoids would be synthesized merely to be utilized for producing the simpler ones. It seems reasonable to assume that the synthesis and degradation of rotenoids are in themselves of some importance in the plant metabolism. It may be that they play a role somewhat similar to that of the respiration chromogens, substances which unite readily with free oxygen under the influence of oxidizing enzymes to produce water and respiration pigments. These pigments are capable of acting as acceptors of hydrogen, to reproduce the original chromogens and oxygen. With gentle oxidation rotenone and deguelin each lose two hydrogen atoms to give dehydrorotenone and dehydrodeguelin: on reduction with hydrogen they add on two hydrogen atoms to give dihydrorotenone and dihydrodeguelin. Neither reaction appears to be reversible *in vitro*, but it is possible under the influence of an enzyme that they act in the plant as hydrogen acceptors, or play some similar simple role. Clark & Keenan (1933) have isolated dehydrodeguelin and dehydrotoxicarol from a *Derris* root which contained no rotenone.

As the rotenoids usually occur mixed with fixed oils in the cotyledons, it is possible that they may take some part in the hydrolysis of these oils during germination. Preliminary experiments were, however, inconclusive.

Work now in progress with interspecific grafts of *Derris* may throw light on the mechanism of rotenoid formation. Thus *D. polystachya*, which contains no rotenoids, has been grafted on to *D. elliptica*, and vice versa. A preliminary result from *D. elliptica* on *D. malaccensis* tends to show that the root of the latter accumulates abnormal amounts of rotenoids as a result of the influence of the scion.

While the evidence suggests that rotenoids perform some useful function in the germinating seeds and young seedlings of these plants,

but may be waste products in all other tissues and are certainly not utilizable as a reserve food supply, it is admittedly exceptional for the same substance to function as a physiologically useful compound and as a waste product, especially in the same plant. The question, therefore, arises whether the rotenoids in the seeds are indeed identical with those in the other tissues. On this point I have some evidence for *Mundulea sericea*. Rotenone is the chief rotenoid extracted from both the stem and the seed and, therefore, these organs contain either rotenone or a rotenone precursor that changes to rotenone during the process of extraction. Since all rotenoids disappear from the seed soon after germination, either rotenone can act as a useful compound in the seed and a waste product in the stem, or one of them, e.g. the seed, contains a rotenone precursor capable of changing to a non-rotenoid during germination, whilst the stem contains rotenone itself or a precursor not normally capable of such a change.

Finally, there is the possibility that other toxic substances that do not respond to Durham's test may occur in some of the tissues, but at present it is not possible to identify any. There is no evidence, however, that any appreciable quantities of such substances occur.

In conclusion, it is an interesting fact that rotenoids are confined to a narrow range of plants: apparently to the two subtribes Tephroseae and Lonchocarpeae, of the different tribes Galageae and Dalbergeae, respectively. As far as I am aware, only species of the genera *Mundulea*, *Tephrosia*, *Millettia*, *Derris* and *Lonchocarpus* contain rotenoids, and not all species of any of these genera contain them. About fifty species are said to be toxic to insects or fish, but the number of rotenoid-containing species is probably higher than this, since some reputed non-toxic ones contain small amounts of rotenoids (e.g. *Tephrosia densiflora*) and many have not yet been examined. The taxonomic distribution of these species within the two subtribes appears to be random, but the general uniformity in the behaviour of rotenoids during germination may be evidence of a single evolutionary origin.

SUMMARY

1. All available parts at all available stages of growth of twenty-two species of Papilionaceae have been examined microscopically for the presence of rotenoids: fourteen contain them, and Table V summarizes all the plant parts in which they occur.

·2. The rotenoid content varies from mere traces in the seeds only of

one species to appreciable quantities in nearly every organ of another species.

3. Seeds of twelve of the rotenoid-containing species were available: all contained rotenoids. It appears to be the rule that if any species contains rotenoids in any tissue it will contain them in its seeds.

4. Roots of thirteen of the species were available: twelve contained rotenoids.

5. In general, rotenoid cells in the roots and stems are normal ones, but in all other tissues they are usually morphologically distinct, and frequently relatively very large.

6. Rotenoids occur in the seeds of species of *Millettia* and *Derris* not in cells but in lysigenous cavities.

7. In many species rotenoids are first detected in the cortex of the root opposite the protoxylem and primary medullary rays.

8. In general, the roots contain rotenoids in the xylem parenchyma, especially in the medullary rays, and in the cortex. In stems they occur mainly in the pith, when not lignified, and also in the phloem, especially in the rays, and in the cortex.

9. At nodes there is frequently an increase in the number of rotenoid cells, especially in the pith.

10. On germination, the seeds of *Mundulea* and *Tephrosia* species at first produce new rotenoid cells, accompanied by an increase in total rotenoid content, in the growing radicle and in the cotyledons. Later, the rotenoids diminish to nil in the radicle and to between 45 % and nil, for different species, in the cotyledons. With *Millettia*, new rotenoid cavities are at first formed in the radicle, and later all rotenoids disappear from it: only a small reduction in the rotenoid content of the cotyledons, however, occurs. With *Derris*, no new rotenoids are formed in the radicle: the amount in the cotyledons in one species diminished to 62 %.

11. Rotenoids in the plumules of *Millettia* and *Derris* species disappear soon after germination.

12. Two instances only of lability of rotenoids have been proved: in the germinating seeds of all species, and in the lignifying pith of the stems of two species.

13. *Derris elliptica* plants placed in the dark until dead show no reduction in the amount of rotenone per plant: indicating that the rotenone is not available as a food reserve.

14. The application of the sap from *Derris* roots and stems to *Derris* cuttings produces no increase in percentage rooting or in the speed of rooting.

15. The results suggest that rotenoids are of some direct use to the germinating seed and seedling, but that they are probably waste products in all other tissues.

Finally, I wish to thank F. J. Nutman for the encouragement and help that he has given me during this investigation and the writing of the paper. I wish also to thank P. J. Greenway for the collecting of some and the naming of much of the plant material used, and H. H. Storey and R. E. Moreau for suggestions and criticism in the writing of this paper, especially the discussion.

REFERENCES

CLARK, E. P. & KEENAN, G. L. (1933). *J. Amer. chem. Soc.* **55**, 422.
EATON, F. M. (1936). *J. agric. Res.* **53**, 442.
GROSS, C. R. & SMITH, C. M. (1934). *J. Ass. Off. Agric. Chem.* **17**, 336.
ROGER, H. P. & CALAMARI, J. A. (1936). *Industr. Engng Chem.* Anal. ed. **8**, 135.
WORSLEY, R. R. & NUTMAN, F. J. (1937). *Ann. appl. Biol.* **24**, 696.

EXPLANATION OF PLATES XXXV–XXXVIII

PLATE XXXV

Fig. 1. $80\,\mu$ transverse section of stem bark of *Mundulea sericea*, showing rotenoid cells, dark, in medullary rays. $25 \times$.

Fig. 2. $80\,\mu$ longitudinal section of stem pith of *Derris elliptica*; rotenoid cells, dark, are seen in chains. $20 \times$.

Fig. 3. $80\,\mu$ transverse section of root bark of *Mundulea sericea*: rotenoid cells dark. $25 \times$.

Fig. 4. $80\,\mu$ longitudinal section of bark of young root of *Mundulea sericea*, showing globular nature of contents of rotenoid cell. $125 \times$.

Fig. 5. $80\,\mu$ transverse section of young root of *Tephrosia Vogelii*, showing rotenoids, black, in the cortex opposite the primary medullary rays, and also in the rays. $37 \times$.

Fig. 6. $80\,\mu$ transverse section of mid-rib of leaflet of *Tephrosia Vogelii*, showing rotenoid cells, black, in the collenchyma. $20 \times$.

Fig. 7. $6\,\mu$ transverse section of *Tephrosia Vogelii* leaflet, showing three large rotenoid cells, white. $125 \times$.

Fig. 8. $6\,\mu$ transverse section of *Tephrosia Vogelii* petal, showing two large rotenoid cells, white. $125 \times$.

PLATE XXXVI

Fig. 1. $6\,\mu$ transverse section of *Mundulea sericea* filament stem, showing one large rotenoid cell containing globules. $150 \times$.

Fig. 2. $6\,\mu$ transverse section of *Mundulea sericea* filament base, showing one large rotenoid cell containing seven globules. $150 \times$.

Fig. 3. $6\,\mu$ transverse section of *Mundulea sericea* anther, showing two large rotenoid cells. $275 \times$.

Fig. 4. $6\,\mu$ transverse section of *Tephrosia Vogelii* anther, showing six large rotenoid cells in the connective tissue. $110 \times$.

Fig. 5. $80\,\mu$ longitudinal section of very small ovule from *Mundulea sericea* bud, showing three rotenoid cells, black. $160 \times$.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 6.

Fig. 8.

Fig. 4.

Fig. 7.
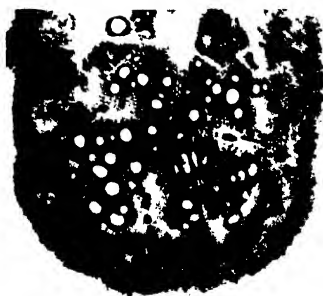
Fig. 5.

Fig. 1.

Fig. 2.

Fig. 4.

Fig. 5.

Fig. 3.
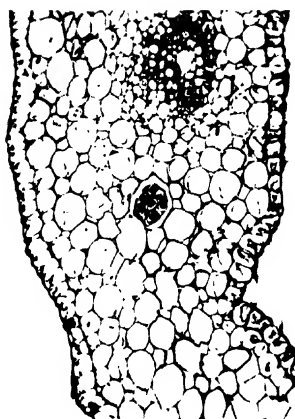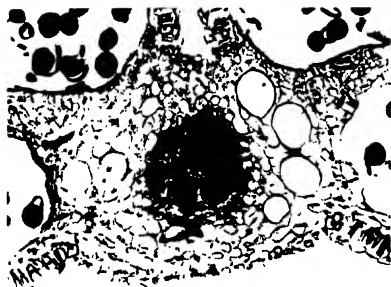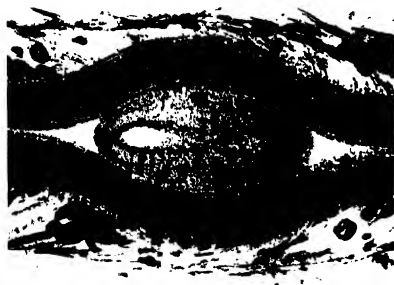
WORSLEY.—The Histology and Physiology of Rotenoids in some Papilionaceae. I (pp. 649-683)

PLATE XXXVII

Fig. 1. 6 μ transverse section of small ovule of *Mundulea sericea*, showing two rotenoid cells. 50 ×.

Fig. 2. 80 μ transverse section of somewhat larger ovule of *Mundulea sericea*, showing rotenoid cells spreading around it. 150 ×.

Fig. 3. 6 μ longitudinal section of part of fairly large ovule of *Mundulea sericea*: note the globular contents of the four rotenoid cells. 150 ×.

Fig. 4. 80 μ transverse section of immature *Mundulea sericea* seed: cotyledons not yet completely differentiated. Rotenoid cells, black, occur only in the testa. 20 ×.

Fig. 5. 80 μ transverse section of ripe seed of *Mundulea sericea*. Rotenoid cells are black. 25 ×.

Fig. 6. 6 μ transverse section of small ovule of *Tephrosia Vogelii*. Large white cells are rotenoid ones. 35 ×.

Fig. 7. 6 μ transverse section of a large rotenoid cell, with globular contents, in *Tephrosia Vogelii* ovule. 200 ×.

Fig. 8. The parenchyma layer of the seed testa of *Tephrosia Vogelii*: rotenoid cells black. 100 ×.

PLATE XXXVIII

Fig. 1. 80 μ transverse section of *Tephrosia Vogelii* ovary: small black cells in the parenchyma of the walls are rotenoid ones. 12 ×.

Fig. 2. 80 μ transverse section of *Tephrosia Vogelii* style, showing rotenoid cells, black, around the periphery. 125 ×.

Fig. 3. 80 μ transverse section of part of *Derris elliptica* seed, showing rotenoid cavities. 17 ×.

Fig. 4. 80 μ longitudinal section of plumule of *Derris uliginosa*, showing rotenoid cavities in the abaxial sides of the immature leaflets. 35 ×.

Fig. 5. *Millettia dura* seed with testa removed, showing rotenoid cavities, white, near the surface. 3 ×.

Fig. 6. 80 μ longitudinal section of plumule of *Millettia dura*, showing rotenoid cavities, black. Note the epidermal expansion opposite the cavities. 45 ×.

Fig. 7. 80 μ transverse section of *Derris elliptica* root grown with calcium deficiency, showing a ring of non-rotenoid cells. 12 ×.

(Received 19 *April* 1939)

STUDIES UPON THE TIME OF FLOWERING OF PLANTS

ANATOMICAL, FLORISTIC AND PHENOLOGICAL ASPECTS OF THE PROBLEM

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(With 9 Text-figures)

CONTENTS

INTRODUCTION

MUCH botanical research of recent years has centred round the causes and control of flowering. Manipulation of temperature is effective for the early blooming of bulbs; variations in photoperiod affect certain other species, whilst many kinds are amenable to vernalization (Whyte & Hudson, 1933). The attainment of a suitable degree of preponderance of carbohydrate over nitrogen in metabolism has been advanced as a physiological cause of flowering. No one of these causes appears adequate to explain the flowering of all kinds of plants, and it becomes increasingly evident that the causes are interrelated. Roberts & Struckmeyer (1938) demonstrated that photoperiodic response in many plants is dependent upon temperature. Grainger (1938) showed that the relative carbohydrate metabolism of late-flowering chrysanthemums is dependent upon photoperiod, though Sheard (1938), working with similar material, found no evidence that it was the carbohydrate-nitrogen ratio in the growing point which was affected.

Any attempt to elucidate the action of various factors upon the time of flowering would, moreover, seem to demand a relation to the time when the flower bud was first formed, and not only to the time when it emerged. It is known that many plants make their flower buds a considerable time before the emergence of bloom. Thus apples (Bijhouwer, 1924; Gibbs & Swarbrick, 1930), pears (Luyten & de Vries, 1926), cherries (Versluys, 1921) and plums (Luyten, 1921) all make flower initials in July or August, but are not covered with blossom until the following May. Lilac, azalea and rhododendron (Luyten & Versluys, 1921) and lily-of-the-valley (Zweede, 1930) make their flower initials in June or July for the following spring. Hyacinth (Blaauw, 1920), tulip Mulder & Luyten, 1928) and daffodil (Huisman & Hartsema, 1933; Grainger, 1935) make flower buds during the summer months, to emerge naturally in the succeeding April, or to be forced into maturity at Christmas. Most of the data which is available refers to trees or to bulbs, which have a specialized organization, and information as to the times when biennials, herbaceous perennials and shrubs make their flower initials is meagre.

The absence of such data militates against a true valuation of any possible photoperiodic response in many plants. Thus the daffodil is a short-day plant when considered from the standpoint of its flower emergence, but a pronounced long-day species if the time of formation of its flower initial be the criterion. It is, moreover, necessary to know more about the times of flower-bud formation before the effects of climate can be evaluated correctly. The present paper gives the results of an investigation into the times of formation of the flower initial in a number of plants of diverse habit, and considers the general effects upon the time of flowering, of such factors as climate and plant form. Further studies upon the relative carbohydrate metabolism of several plants in relation to their organization for flowering will be published later.

THE ANATOMICAL ORGANIZATION FOR FLOWERING

Methods

The general method was to examine at intervals a number of buds or growing points which, by their size, or their position upon the plant, might be presumed suitable for the formation of flower initials. Such buds were dissected separately, and examined under suitable magnification of from 10 to 40 diameters. This was usually sufficient to reveal the nature of the growing point with certainty. A method of retrospective checking was adopted when a considerable number of plants of uniform growth was available. Average counts were made of the number of leaf initials nearer

to the growing point than the smallest expanded leaf. Such leaves were marked in a number of plants which were not dissected until the next examination, when counts were made from the marked leaves. When the flower initial was readily visible it was possible to refer its formation to a time between two previous dissections.

It was sometimes difficult to obtain floriferous plants. Certain patches of wood anemone and blackthorn were marked as floriferous in 1937, but material from them showed no flower buds, nor did they flower, in 1938. It was therefore the practice to examine material from more than one station, as a check, though results given in Tables I–IV are stated for only one particular locality for each species. Variation in the anatomical phenology in different localities in the neighbourhood of Huddersfield was frequently as much as a fortnight, and occasionally slightly more.
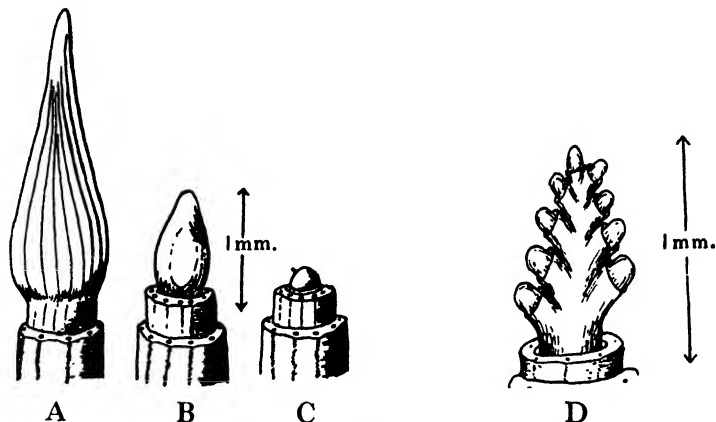
Fig. 1. *Holcus mollis.* A, B and C, dissections of the vegetative growing point, 3 Apr. 1938. D, panicle initial, 24 Apr. 1938.

Results

Dissections of more than a hundred species growing in the vicinity of Huddersfield were made in 1937, 1938 and in the early months of 1939. Some of the most complete results are shown by Tables I–IV, and some of the dissections are further shown by Figs. 1–7. It seemed possible to recognize several types of organization for flowering, and for convenience of reference they are described below.

Classification of types of organization for flowering

A. *Direct flowering plants.*

Development from the first appearance of the flower initial to the open bloom is uninterrupted.

. (i) *Direct flowering from the period of maximum vegetative growth.* Flowers are initiated when leaf growth is at a maximum, and develop

into the complete bloom without interruption. This is the commonest type of floral organization, and results for twenty-eight representative species are given in Table I. Figs. 1–3 further elucidate the transition from vegetative to flower growing points. *Holcus mollis* (Fig. 1) has a terminal flower; *Epilobium angustifolium* (Fig. 2) bears axillary leaf buds at first, but later flowers are produced in the axils of younger leaves; and *Dianthus Allwoodii* (Fig. 3) begins to form its terminal flowers in spring. Certain peculiarities of its growth are discussed later in this paper.

(ii) *Direct flowering from the period of minimum vegetative growth.* Flowers are initiated in late autumn or winter when leaf growth does not take place or is at a minimum. Development into the complete bloom is not interrupted, and this group includes many plants which flower early in the year. Representative species are shown in Table II, whilst Fig. 4 (*Saxifraga tridactylites*) and Fig. 5 (coltsfoot) show actual dissections. *S. tridactylites* is an evergreen, but coltsfoot makes and develops its flowers entirely at the expense of stored food, when no vegetative development of the plant takes place.

5mm.

Fig. 2. *Epilobium angustifolium.* Transition from vegetative buds to flower buds in the axils of leaves, 1 June 1938. The shoot was 2 ft. above ground.

B. *Indirect flowering plants.*

A period of rest intervenes between the formation of the flower bud and its emergence into bloom.

(i) *Indirect flowering from the period of maximum vegetative growth.* Flowers are initiated towards the end of summer, and usually every organ of the future flower is complete in miniature before the leaves fall. Flowers usually emerge in the early months of the succeeding year; most fruit trees belong to this category. Table III shows that several herbaceous perennials, in addition to trees, also adopt this organization for flowering. Fig. 6 illustrates the flower bud of the bilberry, *Vaccinium*

myrtillus, which is notable amongst the species studied for the long period during which the flower bud remains dormant. It can be dissected in a fully formed condition on 17 July, and does not flower until the following
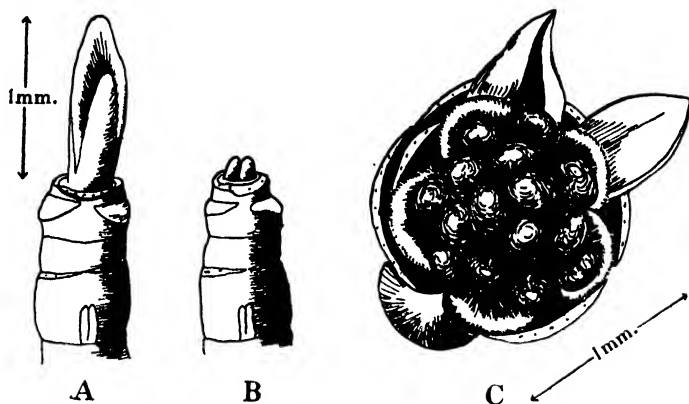
Fig. 3. *Dianthus Allwoodii.* A and B, vegetative growing point, 19 Mar. 1938. C, plan view of flower initial, 17 May 1938.

May. Flower formation probably begins early in June, so that the floral bud takes about 11 months from initiation to emergence. Most fruit trees have a corresponding period of approximately 9 months.
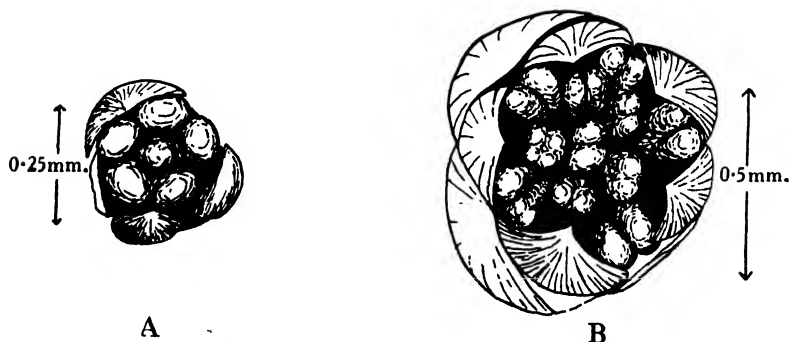
Fig. 4. *Saxifraga tridactylites* Hort. A, plan view of growing point, first initiation of flower, 29 Oct. 1938. B, plan view of growing point with flower initial, 14 Dec. 1938.

(ii) *Indirect flowering from the period of minimum vegetative growth.* Flowers are initiated after the leaves have withered, and after every organ of the future flower is complete in miniature, a period of dormancy

follows. Flowers usually emerge in late winter or early spring, along with the leaves. This type of organization has not been studied in the present investigation, but work upon the bulbs of horticultural value, already cited, have shed much detailed light upon this class.
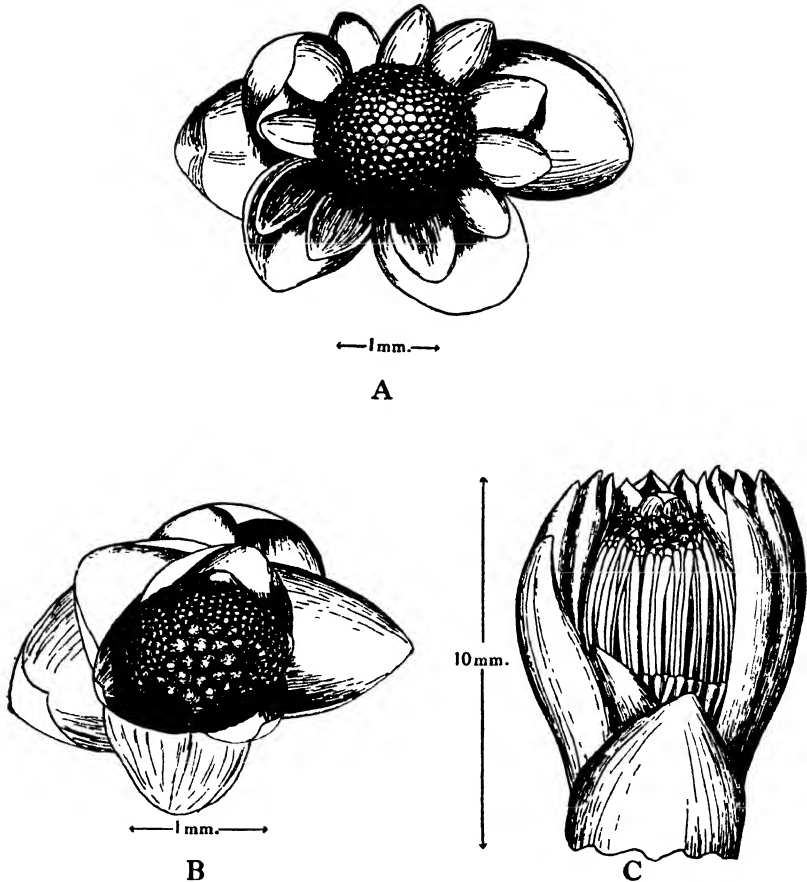
Fig. 5. Coltsfoot, *Tussilago farfara*. A, plan view of the inflorescence initial, 17 Sept. 1938. B, plan view of inflorescence initial, 29 Oct. 1938: 21 disk florets developing. C, side view of inflorescence 3 Dec. 1938. The total length of the inflorescence, shown as 10 mm. on 3 Dec., had increased to an average of 17 mm. on 1 Jan. 1939, when the florets also appeared yellow.

C. *Cumulative flowering.*

Flower initials are produced in regular succession, and all emerge together. Tables IV and V, and Fig. 7 illustrate this behaviour. The plantains and dandelion are all examples of long-term preparation for a period of maximum flowering. Flowering initials are formed in the axils of the rosette leaves, beginning about November for the dandelion and *Plantago lanceolata*, and in early spring for *P. major* and *P. media*. The
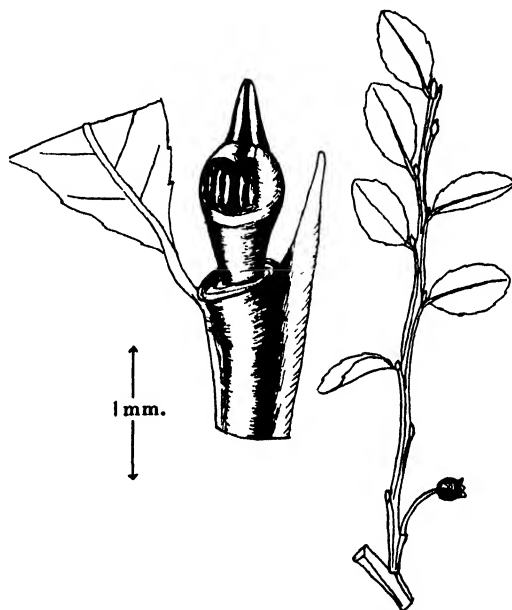
Fig. 6. Bilberry, *Vaccinium myrtillus*. Dissection of flower initial from terminal bud of the adjoining shoot, 17 July 1938.

inflorescences all emerge together at the respective times shown in Table IV for each species. Table V and Fig. 7 show the relative sizes of inflorescence initials in *P. major* on 22 May 1938; they all emerged together about 25 June. Vegetative buds appear in the axils of a few leaves, and proliferate to form the clumps characteristic of these plants. *P. lanceolata* and dandelion make their inflorescence buds largely during the period of presumed minimal photosynthesis, whilst *P. major* and *P. media* are florally determined when leaf growth is vigorous, thus suggesting the same subdivisions as are mentioned above for direct- and indirect-flowering plants.
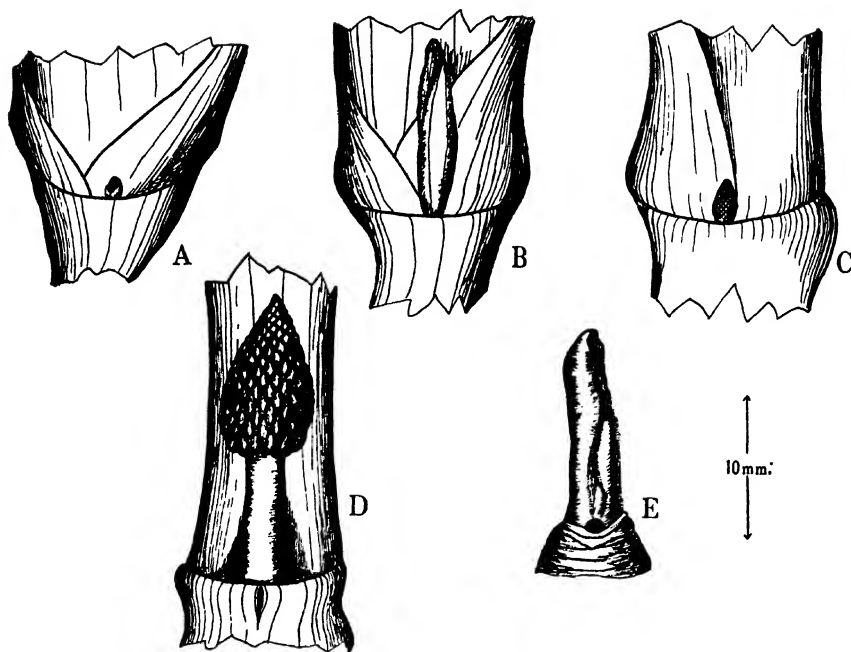
Fig. 7. Broad-leaved plantain, *Plantago major*. Leaf buds and inflorescence initials referred to in Table V. The base of the subtending leaf is shown pressed away from the main shoot in each case. A, outermost vegetative bud. B, innermost vegetative bud. C, outermost inflorescence bud. D, largest inflorescence bud. E, innermost inflorescence bud.

D. *Climax flowering.*

The plant is entirely vegetative for a number of years, and then entirely floriferous for a year, e.g. *Amorphophallus titanum* in the Hamburg Botanical Garden. This species is vegetative for about 40 years, and then sends forth a flower. Some species of agave behave similarly.

The chief significance of these results lies in Tables II and IV. Many plants have been found (Table II) which begin to make their flower initials when vegetative growth is at a minimum (e.g. saxifrage) or when there is no leaf growth (e.g. coltsfoot). It would appear difficult to align this behaviour, and that of § B (ii) above (bulbs), totally with any external cause. Cumulative flowering (Table IV) is probably a direct response to temperature. Preliminary experiments with *Plantago lanceolata* suggest that the inflorescence buds can be made to emerge in

March if the temperature is raised artificially to the average of May, when flowering usually takes place. This further suggests that flower initiation and emergence are separately favoured by different external conditions.

Table I. *Direct flowering from the period of maximum vegetative growth. Times of flower bud formation and of flowering*

Name of species	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Aira flexuosa L.	.	.	.	− 3	− 6 +12	*30	.	.	.	.	.	.
Angelica sylvestris L.	.	.	−17	−24	−15	+ 6	+	*4	.	.	−12	.
Calceolaria polyrhiza Hort.	.	.	.	− 3	+16	*20	.	.	.	.	.	.
Calluna vulgaris Salisb.	.	.	.	−10	−14	+30	+	*20	.	.	.	.
Campanula rotundifolia L.	.	.	− 3	−24	.	−11	+ 6 *24	.	.	.	.	.
Carduus lanceolatus L.	.	.	.	− 3	−16	+ 6	*15	.	.	.	.	.
C. palustris L.	.	.	.	−24	−14	+ 6	*15	.	.	.	.	.
Centaurea nigra L.	.	.	.	− 9	−20	+ 6	+	*13	.	.	.	.
Convolvulus sepium L.	.	.	.	−24	+15	*25	.	.	.	.	.	.
Digitalis purpurea L.	−8	.	− 3	+ 3	+ 7	*25	.	.	.	.	.	.
Epilobium angustifolium L.	.	.	−31	−17	− 7	+ 1	*4	.	.	.	.	.
Erica cinerea L.	.	.	.	−10	.	−12	+26	*20	.	.	.	.
Hedera helix L.	.	.	.	.	−11	− 6	+15	+	*20	.	.	.
Heracleum sphondylium L.	.	.	.	−24	+ 7	+	*6	.	.	.	.	.
Hieracium pilosella L.	.	.	.	− 6	.	.	*2	.	.	.	.	.
H. boreale Fr.	.	.	−31	.	.	.	*4	.	.	.	.	.
Holcus mollis L.	−2	.	.	− 3 +24	+	+	*6	.	.	.	.	.
Mentha sativa Hort.	.	.	.	.	−10	+25	+	*20	.	.	.	.
Rosa canina L.	.	.	−12	+24	+ 7	*25	.	.	.	.	.	.
Rumex obtusifolius L.	.	.	− 2	−24	+ 1	*25	.	.	.	.	.	.
Scabiosa succisa L.	.	.	.	− 9	−28	+22	+	*13	.	.	.	.
Sedum acre L.	.	.	.	− 3	+20	*30	.	.	.	.	.	.
S. anglicum Huds.	.	.	.	− 3	+20	+	*17	.	.	.	.	.
S. spectabile Hort.	.	.	.	− 3	+20	+	*10	.	.	.	−16	−14
Stachys betonica Benth.	.	.	.	− 9	−17	.	.	*12	.	.	.	.
Teucrium scorodonia L.	.	.	.	−10	−17	.	*15	.	.	.	.	.
Urtica dioica L.	.	.	−16	+ 9	+28	+	*12	.	.	.	.	.

The numbers represent the days of the months under which they occur. + and − refer to flower initials, and * denotes the time of first flowering or maximum flowering. Thus " − 17" under the month of April means that no flower initial could be found on 17 April; " +15" under July indicates that flower initials were observed on 15 July, and "*20" under August means that the species was first observed to flower on 20 August.

Each result is the product of a number of dissections, usually not less than six, and if one dissection yields a flower bud, the result is shown as + in the table. Plus signs without numbers are included with some species to indicate continuity. Thus in *Hedera helix* flower initials which can be found on 15 July would ultimately form the complete flowers which emerge on 20 September.

ABNORMAL FLOWERING IN AUTUMN

The very mild autumn of 1938 provided opportunity for the observation of numerous plants which flowered out of their usual season. Table VI enumerates twenty-eight typical species which were observed personally or were reported in response to an appeal for information in

Table II. *Direct flowering from the period of minimum vegetative growth. Times of flower bud formation and of flowering*

Name of species	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Anthriscus sylvestris Hoffm.	−10	+25	+31	+	*20	.	.	.	.	.	.	.
Chrysanthemum leucanthemum L.	.	−26	+12	+16	+	*15	.	.	.	.	.	.
Dactylis glomerata L.	−10	+26	+	+10	+	*10	.	.	.	−29	.	.
Dianthus Allwoodii Hort.	−17	−8	−19	+1	+17	*16	.	.	.	.	−16	−3
Lonicera peri-clymenum L.	−6	.	+12	+9	+	+	+	*10	.	.	.	−10
Lupinus polyphyllus Hort.	+12	+20	+	+9	*22	.	.	.	.	.	−5	+11
Lychnis dioica L.	−1	+26	+9	+24	*28	.	.	.	.	.	.	.
Ranunculus repens L.	−8	−11	+2	+	*15	.	.	.	.	.	.	.
Rubus fruticosus L.	−14	−11	+9	+7	+	+	*30	.	.	−14	−12	.
Rumex acetosa L.	−8	.	+12	+3	*30	.	.	.	.	.	.	.
Saxifraga tridactylites Hort.	+9	+20	+1	+	*11	−14	.	.	−20	−10	+16	+14
										+29		
S. hypnoides Hort.	+28	+	+1	+	*7	.	.	.	.	−29	−16	+3
Sisymbrium alliaria Scop.	+17	+22	+21	*24	.	−6	−20	−1	.	−29	.	−3
Tussilago farfara L.	+2	+	*3	.	.	.	−12	−10	+17	+14	+	+1

For explanation, see Table I.

Table III. *Indirect flowering from the period of maximum vegetative growth. Times of flower bud formation and of flowering*

Name of species	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Anemone nemorosa L.	+5	+	+	*20	.	.	−20	+5	+	+	+20	+10
Arabis hirsuta Hort.	+	+12	+	+	*27	.	.	.	+28	+14	+	+17
Auricula sp. Hort.	+7	*6	.	.	.	.	.	.	+24	+17	+11	+14
Eriophorum angustifolium Roth.	+	+27	.	+	*17	.	.	.	+14	+14	+	+
E. vaginatum L.	+	+	+	*29	.	.	.	.	.	+14	+	+
Fragaria vesca Hort. Royal Sovereign	+	+2	+	+3	+	*15	.	−1	.	+23	+	+
Ilex aquifolium L.	+	+	+	+	+	*17	.	+15	+6	+	+	+
Primula wanda Hort.	+	+	+	*1	.	.	.	.	.	+1	+	+
P. veris Hort.	+	+	+	+	*10	.	−15	+19	+	+1	+	+
Salix caprea L.	+	+	*11	.	.	−15	+9	+	+12	+	+	+
Vaccinium myrtillus L.	+	+27	+	+	*6	.	+17	+	+	+	+	+16

For explanation, see Table I.

Table IV. *Cumulative flowering. Times of flower bud formation and of flowering*

Name of species	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Plantago lanceolata L.	+	+17	+31	+	*15	.	.	.	.	.	+8	+10
P. media L.	.	.	.	+23	+7	+	*2	.	.	.	.	.
P. major L.	.	.	−7	+12	+22	*25	.	.	.	.	.	.
Taraxacum officinale Weber	+	+	+	*30	.	−28	.	.	.	.	+9	+

For explanation, see Table I.

Table V. *Relative sizes of leaves and flower initials in* Plantago major,
on 22 *May* 1938, *four weeks before flowering*

Leaf no. ...	1	2	3	4	5	6	7	8	9	10	11	12	13
Length in mm.	100	165	168	198	225	232	250	223	202	127	84	49	15
Nature of bud in axil	—	Veg.	Veg.	Veg.	Veg.	Fl.	Fl.	Fl.	Fl.	Fl.	Fl.	Fl.	Fl.
Length in mm.	—	1·5	1·5	2·0	12·0	1·5	3·0	22·0	8·0	5·0	2·5	1·0	0·5
Drawing in Fig. 7 labelled		A			B		C	D					E

All the inflorescences emerged together, about 25 June. Leaf no. 1 was outermost.

The Manchester Guardian, 22 September 1938. The species had emergent
or opened bloom before the end of November. Many species in the table
normally flower for a period during the summer, making their flower
initials singly or at intervals, and developing them into open bloom.
Their late blooming is but an extension of normal behaviour, in response
to the summer-like climate of the autumn. The species marked * in
Table VI, however, are known to make their flower buds in late summer,

Table VI. *Plants flowering abnormally between September and*
November 1938

Apple (Lane's Prince Albert)*	Honesty
Auricula*	Laburnum*
Berberis stenophylla	Lupin
Brompton stock	Magnolia
Broom (*Cytisus europeus*)*	Ox-eye daisy (*Chrysanthemum leucanthemum*)
Campanula garganica	Polyanthus (*Primula variabilis*)*
Cotoneaster microphylla	Primrose (*Primula vulgaris*)*
Cowslip (*Primula veris*)	Pyrethrum
Daphne Mezereum	Raspberry*
Delphinium	Rhododendron*
Doronicum	Rose (garden)
Erigeron (garden)	*Salix caprea**
*Forsythia suspensa**	*Trollius europeus*
Helleborus foetidus	Wallflower

Species marked * make their flower buds in late summer, for emergence the following
spring (see text).

for emergence in the following spring. Abnormal flowering in this case
represents the precocious development of flower initials already present;
they emerged in September, October or November, instead of the
following April or May. It may, therefore, be concluded that the dormant
period or essential rest period, if any, of the flower bud in these species
is physiologically concluded before November. The normal extension of
the rest period until April or May of the following year must therefore be
due, either wholly or largely, to the effects of climate.

The open weather of autumn 1938 also appeared to affect the relative
stage of development of flower initials which did not actually emerge.

Thus inflorescences of *Eriophorum angustifolium* were, on an average, 2·2 cm. long on 27 December 1937, but were 9 cm. long on 12 November 1938. This observation is in agreement with results published for bulbs (e.g. Hartsema *et al.* 1930, 1932).

THE EFFECT OF PLANT HABIT UPON THE TIME OF FLOWERING

It might be thought that the habit of a plant could exert an influence upon the time of flowering. Thus biennials and perennials, which often have a store of food, might perhaps be expected to flower earlier than annuals, which need to make all their food in the same short season in which they flower. Table III shows that some herbaceous perennials can make their flower initials in the year before they emerge. This question was investigated statistically, by the preparation of frequency curves showing the number of species flowering in each month of the year. The British flora, as set forth in the *Botanist's Pocket Book* of Hayward & Druce (17th ed.), was so treated, and the plant forms and times of flowering are those specified in that volume.

The results are shown graphically in Fig. 8, and reveal a maximum number of species flowering in July, for all plant habits except corms, bulbs, trees and shrubs. Bulbs and corms have stores of food, and show maxima between April and May, and in September. Their avoidance of growth in summer is perhaps a reaction of their limited root systems to relative drought at that time. They show the same relation to season in the southern hemisphere, and do not flower at the actual times which were suitable in the northern half of the globe. Periodicity must, in fact, be shifted about half a year when bulbs are taken from the northern to the southern hemisphere (Hartsema & Blaauw, 1935).

Trees reveal a maximum in May, shrubs in June, and herbaceous perennials in July. This suggests that total size of plant, with its corresponding store of food, can affect the time of flowering. A consideration of fifty-two herbaceous perennials about which data has been accumulated also confirms the view that relative food supply is linked with time of flowering. Eighteen of the fifty-two species flowered in the month of May or before; all began to form their flower initials in the previous year, and all were either evergreen, or had stores of food in rhizomes or taproot. Thirty-four species flowered in the month of June or later. Twenty-six of these were only represented in winter by a dormant crown with fine roots, that is they had little stored food. Seven species had stores of food, and one was evergreen. All thirty-four species formed their flower
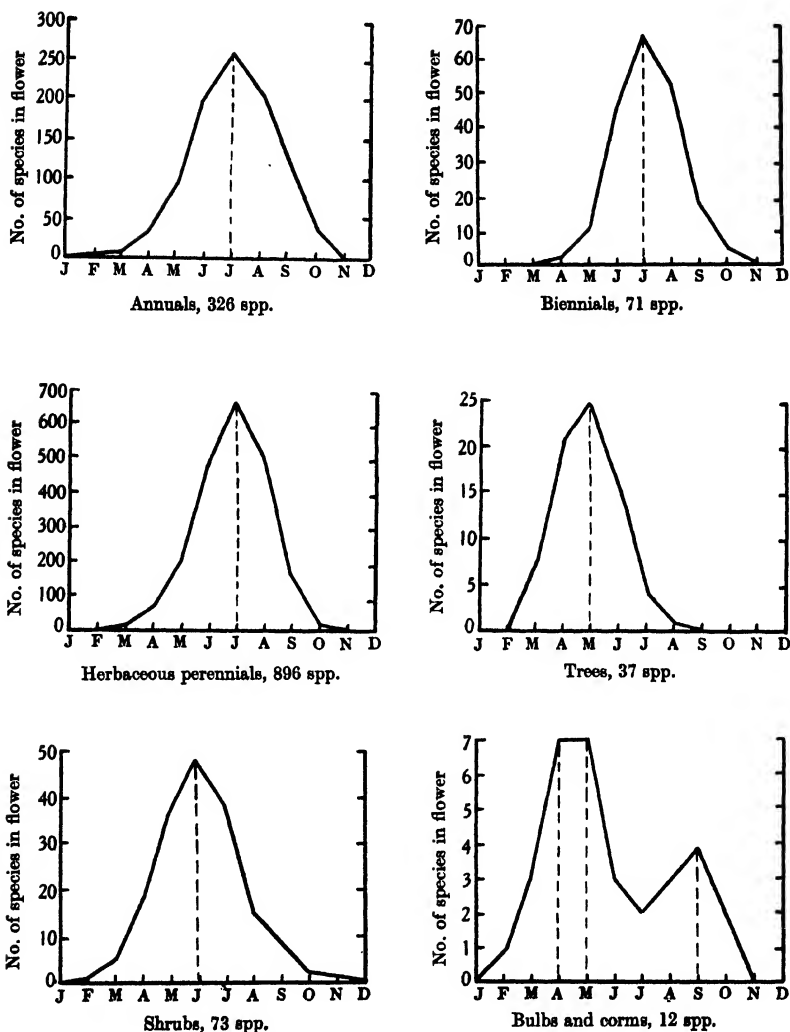
Fig. 8. Frequency diagrams for the flowering of plants of varying habit included in the British flora. Horizontal scales represent months of the year.

buds in the current year. It therefore appears that plants which are evergreen or have stores of food do not necessarily flower early, but that in order to bloom in the earlier months of the year a plant must either have stores of food, or bear functional green leaves through the winter, and must also initiate flower formation some time in the previous year or during the winter season. Fig. 8 suggests that the majority of wild herbaceous perennials and biennials of the British flora have, in general, inadequate stores of food for early flowering. They have no advantage over annuals, so far as their habit *per se* is concerned.

The question of food supply also appeared to exert an effect upon the flowering of *Dianthus Allwoodii*. Unsightly clumps of this plant, trimmed in January 1936 and 1937, failed to flower in the following seasons, while untrimmed clumps flowered profusely. It was thought that trimming had removed flower initials already made, but subsequent dissections showed that they were not present until the end of March (Table I and Fig. 3). Trimming removed the larger shoots, and left only small ones, which could evidently not form flower initials when the proper time arrived, an inability most probably due to insufficient food.

THE EFFECT OF CLIMATE PRIOR TO FLOWER EMERGENCE

Researches upon the treatment of various bulbs for early flowering have shown that temperatures at all periods between the initiation of flower formation and blooming may affect the time at which the flower emerges (Blaauw *et al.* 1932; Hartsema *et al.* 1930, 1932). Little work appears to have been done in this connexion with other plants, but the Royal Meteorological Society has maintained a large-scale survey of the phenology of thirteen wild plants since 1891, and fourteen since 1925 (Mawley, 1891–1910; Clark *et al.* 1911–34). This mass of data, with its corresponding figures for temperature, has been used to prepare a series of correlation diagrams of the two variables, temperature and time of flowering. Correlations have been prepared for each species for every month of the year, and for several meteorological districts of the British Isles. Typical diagrams, for coltsfoot in the Midlands, are portrayed by Fig. 9, whilst a larger number of results is shown in Tables VII and VIII. Correlation of these results with times of formation of the flower initials suggests the important conclusion that temperatures *prior to*, as well as after, the initiation of flower bud formation in some plants, e.g. ivy, coltsfoot and others, exert an effect upon the time of flowering. It is most noticeable that temperatures during some months (e.g. July to October
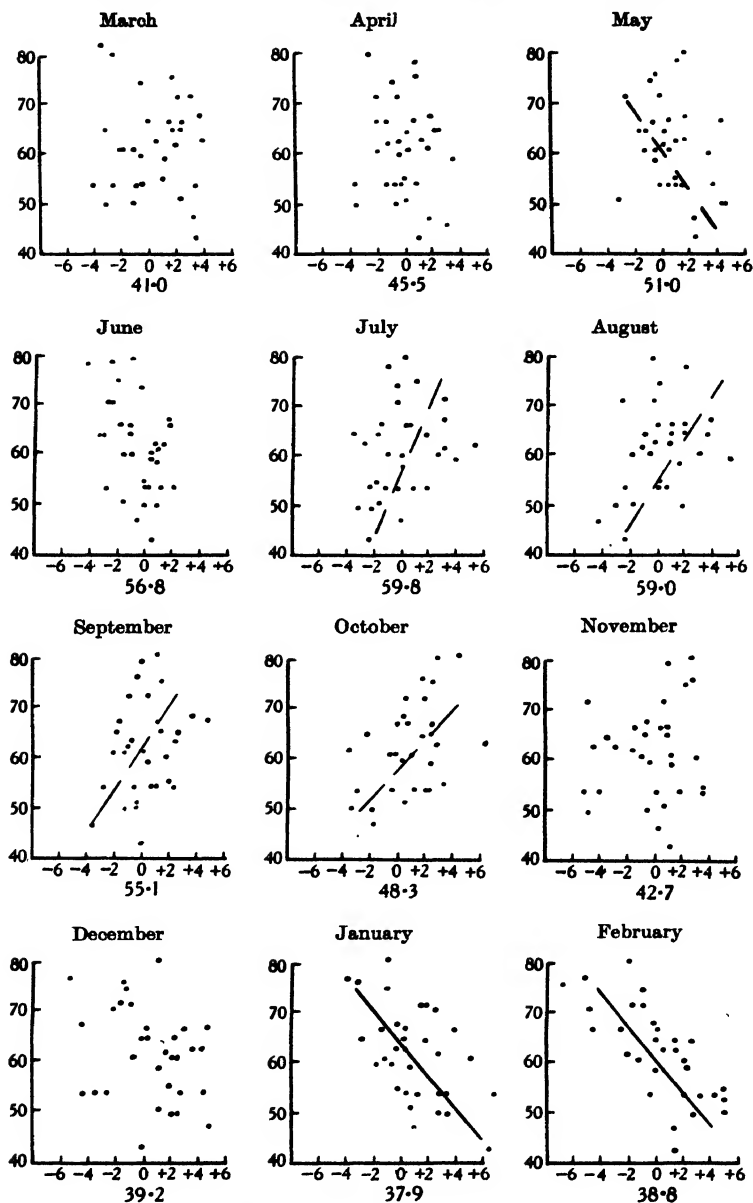
Fig. 9. (For legend see p. (

Table VII. A. *Qualitative temperature correlations for the Midlands of England.*
B. *Approximate time of flower bud formation and of flowering*

		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Hazel	A	\		...	...	...	\	...	...	...	...	...	...
	B	+	*				−	+	+	+	+	+	+
Coltsfoot	A	\	\	...	...	\	...	/	/	/	...	...	
	B	+	*				−	−	−	+	+	+	+
Wood anemone	A	...	\	\	...	...	...	/	/	/	/	...	...
	B	+	+	*			−	+	+	+	+	+	+
Blackthorn	A	\	\	...	...	...	...	...	...	...	...	...	...
	B	+	+	*			−	−	+	+	+	+	
Garlic hedge-mustard	A	...	\	\	\	...	...	...	...	...	...	...	...
	B	+	+	+	*			−	−	−	−		
Horse chestnut	A	...	\	\	\	\	\	...	...	...	...	...	...
	B	+	+	+	*	−	−	+	+	+	+	+	+
Hawthorn	A	...	\	\	\	...	\	...	...	...	...	...	...
	B	+	+	+	*		−	−	+	+	+	+	
White ox-eye daisy	A	...	\	\	\	*	...	...	...	...	...	...	...
	B		−	+	+	*							
Dog rose	A	...	\	\		*	...	...	...				
	B			+	*								
Black knapweed	A	...	...	...	...	\	\	...	...	...	...	...	...
	B					−	+	*					
Harebell	A	...	...	...	...	...	\	...					
	B						−	*					
Greater bindweed	A	...	...	...	...	\	\	...	...	...	...	...	...
	B					−	+	*					
Devil's-bit scabious	A	...	...	...	...	...	...	\	...	...	...	...	...
	B			−	−	−	+	*					
Ivy	A	...	...	...	...	\	\	...	...	\	...	...	...
	B					−	−	+	+	*			

The symbols \ and / indicate the trend of the dots in the correlation diagrams. \ means that flowering is hastened by temperatures warmer than the average, and / that bloom emergence is hastened by cooler temperatures than normal. The symbol ... shows that a correlation diagram has been prepared, but reveals no effect of temperature, i.e. the dots of the correlation diagram are either random, or their trend is parallel to one axis. * indicates flowering, + that a flower initial or bud is present, − that no flower bud has been observed. Averages for 1902–34. An actual series of correlation diagrams for coltsfoot is shown in Fig. 9.

Fig. 9. Correlations between the two variables, temperature and time of flowering, for the coltsfoot in the Midlands of England. The vertical scale of each diagram represents the time when flowering took place, counting the number of days from 1 Jan. 50, for instance, is 19 Feb., the 50th day of the year. The horizontal scales give temperature variations from the average "0", the actual values for which are stated in °F. for each month, below the zero. The dots portray data from 1902 to 1934, one dot for each year. Trends of the correlations are shown by oblique lines in the months of May, July to Oct., Jan. and Feb. A line sloping forward (/) indicates that flowering (in the succeeding Feb. or Mar.) is retarded by temperatures higher than normal, whilst a line sloping backward (\) means that flowering is hastened by warmer temperature. Thus higher temperatures than the average in May, in Jan. and Feb., and lower temperatures than the average, from July to Oct., hasten the appearance of the flower in the following Feb. or Mar. Leaf formation of coltsfoot in the Midlands begins in Mar., and persists until Sept.; flower buds first appear in mid-Sept., and emerge in the following Feb. or Mar.

for coltsfoot in the Midlands of England) are too high for the quickest emergence of the flower in the subsequent season. Data for the north of Scotland are somewhat scanty, but it is perhaps significant that no such temperature correlations are there observable for coltsfoot in the late summer months. The naturally lower temperatures in Scotland at this time appear to be more suited to the needs of this plant.

Table VIII. A. *Qualitative temperature correlations for the south-west of England.*
     B. *Approximate times of flower bud formation and of flowering*

		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Hazel	A	\				...	\	...	...	...	...	...	...
	B	*					−	+	+	+	+	+	+
Coltsfoot	A	\	\			...	...	/	/	/	...	...	...
	B	+	*				−	−	−	+	+	+	+
Wood anemone	A	...	\	\		...	...	/	/	/	/	...	...
	B	+	+	*			−	+	+	+	+	+	+
Blackthorn	A	...	\	\			...	...	...	...	...	...	...
	B	+	+	*			−	−	+	+	+	+	+
Garlic hedge-mustard	A	...	\	\	\		...	...	/	/	/	...	...
	B	+	+	+	*		−	−	−	−	−	−	−
Horse chestnut	A	...	\	\	\		...	...	/	...	...	...	...
	B	+	+	+	*		−	+	+	+	+	+	+
Hawthorn	A	...	\	\	\		...	...	...	...	/	/	...
	B	+	+	+	*		−	−	+	+	+	+	+
White ox-eye daisy	A	...	\	\	\	...	...	...	...	...	...	...	...
	B		−	+	*								
Dog rose	A	...	\	\	\	\	...	...	...	...	...	...	...
	B		−	+	+	*							
Black knapweed	A	...	...	...	...	\	...	...	...	...	...	...	...
	B			−	+	*							
Harebell	A	...	...	...	...	...	\	...	...	...	...	...	...
	B						*						
Greater bindweed	A	...	...	...	...	\	\	...	...	...	...	...	...
	B				−	+	*						
Devil's-bit scabious	A	...	...	...	...	...	...	\	...	...	...	...	...
	B					−	+	*					
Ivy	A	...	...	...	...	\	...	...	...	...	...	...	...
	B						−	−	+	+	*		

For explanation, see Table VII. Averages for 1909–34.

Another feature of Fig. 9 and Tables VII and VIII is the pronounced effect of temperatures higher than the average in the months immediately before flower emergence. This effect is seen 3 months before blooming in many species, and 4 months before in the dog rose. Experimental evidence has also been obtained for the hastening of daffodil bloom emergence under conditions warmer than normal (Grainger & Crawshaw, 1939).

The results suggest that it should be possible to exercise horticultural control of temperature both prior to, and during, flower-bud initiation, and also for a time before flower emergence.

DISCUSSION

The chief results established by the present investigation are that many plants begin to form their flower initials during the period of minimal vegetative growth (Table II), and that temperatures *prior to* the floral differentiation of the growing point can affect the time of flower emergence (Tables VII and VIII). The latter finding might conceivably be explained upon the assumption that the temperatures affected the relative rate of photosynthesis, and therefore the relative amounts of food available when the flower initials began to be formed. Times of flower emergence were not always advanced by temperatures higher than normal; the reverse was often the case. This fact does not militate against the idea of a direct causal relation through the vigour of photosynthesis, since more carbohydrate may be translocated from leaves at the lower temperature than from leaves choked with carbohydrate under the warmer conditions. Initiation of the flower in the depth of winter, however, does not seem to bear any relation to external influences.

Some plants which flower indirectly from the period of maximum vegetative growth can bloom abnormally in a mild autumn. Their physiological rest period, if any, would seem to be concluded before this time, and the normal extension of dormancy until the following spring is apparently a direct effect of winter climate. This should be correlated with statements by Chittenden (1939) that development of the young flower in such plants continues slowly for a time into the autumn. The rest period may possibly be much shorter than is commonly supposed.

Cumulative flowering is interpreted as a direct response to temperature; flower buds formed in regular succession all emerge together when the temperature becomes sufficiently high.

Plant habit does not appear to affect the time of flowering, unless associated with a store of food, but flower emergence in thirteen wild plants is hastened by a temperature higher than that of normal climate at the time of opening (Tables VII and VIII). This effect can often be seen up to 3 months before blooming, e.g. blackthorn, garlic hedge mustard, horse chestnut, hawthorn, white ox-eye daisy, and dog rose. Experimental evidence of the hastening of daffodil bloom emergence by temperatures higher than normal has been obtained by Grainger & Crawshaw (1939). It would appear from the data here presented that in many plants, flower bud formation and flower emergence are governed by separate conditions of temperature.

The general conclusion from these results is that flower formation and emergence are part of an innate rhythm which can be affected to a certain limited extent by external conditions at various times. It does not seem possible to align the data here presented with any cause of flowering entirely or largely dependent upon external factors, nor with the usual conception of the carbohydrate-nitrogen ratio hypothesis. The one consistent channel of evidence is that flowering cannot take place unless sufficient food is available. Thus, it appears that a plant is not likely to flower early in the year unless it is evergreen or has stored food. It must, moreover, usually begin the formation of its flower initial in the previous year or during the winter. The removal of large shoots of *Dianthus Alwoodii* 3 months before the time when flower initials should be initiated prevented their formation. This is interpreted as the removal of food in the shoots below the level of that required for the formation of flower buds. The effect upon the time of bloom emergence of temperature prior to flower initiation is likely to be an influence upon food manufacture. Since food stored in underground vegetative organs can be adequate for the entire needs of flowering, as in coltsfoot, it seems likely that it is, at the outset, sufficient carbohydrate food that is required (cf. also Sheard, 1938), though a certain general balance of nutrients would probably be necessary for the quickest accomplishment of floral growth. Given adequate supplies of food, a plant can flower early (though not all species with stored food do so), but limited food prevents early blooming.

SUMMARY

1. The growing points of more than 100 plant species of diverse habit have been examined between 1937 and 1939, in order to find the exact times of flower initiation.

2. Types of organization for flowering may be classified into (*a*) direct-flowering plants, (*b*) indirect-flowering plants, (*c*) cumulative-flowering plants and (*d*) climax flowering plants. Initiation of the flower in (*a*), (*b*) and (*c*) may either begin (i) during the period of maximum vegetative growth or (ii) when vegetative growth is at a minimum. In the latter class, any direct cause of flowering operated entirely or largely by external influences, such as those affecting the vigour of photosynthesis, would appear to be eliminated.

3. Abnormal flowering of some indirect-flowering plants during the autumn of 1938 suggests that their physiological rest periods were

concluded at that time. Their normal extension of dormancy until the following spring is probably a direct effect of winter climate.

4. Plant habit, as annual, biennial, perennial, does not appear to affect the time of flowering, unless associated with a store of food, but not all plants with stored food flower early.

5. A series of correlation diagrams of the two variables, temperature and time of flowering, has been prepared from the Royal Meteorological Society's phenological data. These suggest that temperatures *before* the initiation of flower bud formation, in addition to those at other periods of floral development, may affect the time of flowering. Some species appear to be hastened by higher temperatures than normal at this period, e.g. ivy, and yet other kinds by temperatures lower than normal, e.g. coltsfoot.

6. Flower emergence in thirteen wild plants is hastened by a temperature higher than that of normal climate for a period just before the time of opening.

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REFERENCES

BIJHOUWER, J. (1924). De periodiciteit van de knopentwikkeling bij den appel. *Meded. LandbHoogesch., Wageningen,* **27**, 9.

BLAAUW, A. H. (1920). Over de periodiciteit van *Hyacinthus orientalis. Meded. LandbHoogesch., Wageningen,* **18**, 3.

BLAAUW, A. H., HARTSEMA, ANNIE M. & HUISMAN, EBELINE (1932). Temperatuur en Strekkingsperiode van de *Narcis.* I. *Proc. Acad. Sci. Amst.* **35**, 6.

CHITTENDEN, F. J. (1939). The behaviour of plants. I. *J. R. hort. Soc.* **64**, 73–82.

CLARK, J. E. *et al.* (1911–34). Annual reports upon the phenology of the British Isles. *Quart. J. R. met. Soc.* as follows:

Clark, J. E. & Hooker, R. H. (1911–13).

Clark, J. E. (1914).

Clark, J. E. & Adames, H. B. (1915–20).

Clark, J. E., Adames, H. B. & Margary, I. D. (1921).

Clark, J. E., Margary, I. D. & Marshall, R. (1924–6).

Clark, J. E., Margary, I. D., Marshall, R. & Cave, C. J. P. (1927–9).

Clark, J. E., Margary, I. D., Marshall, R., Cave, C. J. P. & Bonacina, L. C. W. (1930).

Clark, J. E., Margary, I. D., Marshall, R. & Cave, C. J. P. (1931–4).

GIBBS, MARGARET A. & SWARBRICK, T. (1930). The time of differentiation of the flower bud of the apple. *J. Pomol.* **8**, 1.

GRAINGER, J. (1935). Production of early daffodil bloom. *Gdnrs' Chron.* March 9.

—— (1938). The relation of nocturnal translocation to the time of flowering. *Ann. appl. Biol.* **24**, 1–19.

GRAINGER, J. & CRAWSHAW, F. (1939). The effect of temperature upon daffodil flower buds. *Gdnrs' Chron.* 4 Feb.

HARTSEMA, ANNIE M. & BLAAUW, A. H. (1935). Verschuiving der periodiciteit door hooge temperaturen. *Proc. Acad. Sci. Amst.* **38**, 7.

HARTSEMA, ANNIE M., LUYTEN, IDA & BLAAUW, A. H. (1930). De optimale temperaturen van bloemaanleg tot bloei. *Verh. Akad. Wet., Amst.,* Afd. Natuurkunde, 2de Sect. **27**, no. 1.

—— —— —— (1932). De optimale temperaturen van bloemaanleg tot bloei voor *Hyacinthus orientalis. Verh. Akad. Wet., Amst.,* Afd. Natuurkunde, 2de Sect. **29**, no. 5.

HUISMAN, EBELINE & HARTSEMA, ANNIE M. (1933). De periodieke ontwikkeling van *Narcissus pseudonarcissus.* I. *Meded. LandbHoogesch., Wageningen,* **37**.

LUYTEN, IDA (1921). De periodiciteit van de knopentwikkeling bij den pruim. *Meded. LandbHoogesch., Wageningen,* **18**, no. 4.

LUYTEN, IDA & VERSLUYS, MARTHA C. (1921). De periodiciteit van de knopontwikkeling bij Rhododendron, Azalea en Syringa. *Meded. LandbHoogesch., Wageningen,* **22**, no. 6.

LUYTEN, IDA & DE VRIES, E. (1926). De periodiciteit van de knopontwikkeling bij den Peer. *Verh. Akad. Wet., Amst.,* Afd. Natuurkunde, 2de Sect. **24**, no. 4.

MAWLEY, E. (1891–1910). Annual reports upon the phenology of the British Isles. *Quart. J. R. met. Soc.*

MULDER, R. & LUYTEN, IDA (1928). De periodieke ontwikkeling van de Darwin-tulp. *Verh. Akad. Wet., Amst.,* Afd. Natuurkunde, 2de Sect. **26**, no. 3.

ROBERTS, R. H. & STRUCKMEYER, ESTHER (1938). The effects of temperature and other environmental factors upon the photoperiodic responses of some of the higher plants. *J. agric. Res.* **56**, 633–78.

SHEARD, G. F. S. (1938). Thesis, Leeds University.

VERSLUYS, MARTHA C. (1921). De periodiciteit van de knopontwikkeling bij den Kers. *Meded. LandbHoogesch. Wageningen,* **18**, no. 4.

WHYTE, R. O. & HUDSON, P. S. (1933). Vernalization. *Bull. Imp. Bur. Pl. Genet.* no. 9.

ZWEEDE, A. K. (1930). De periodieke ontwikkeling van *Convallaria majalis. Verh. Akad. Wet., Amst.,* Afd. Natuurkunde, 2de Sect. **27**, no. 2.

INTRANUCLEAR INCLUSIONS IN VIRUS INFECTED PLANTS

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(With Plate XXXIX)

INTRODUCTION

CHARACTERISTIC intracellular inclusions, both crystalline and amorphous, have been described in plants and animals infected with different viruses. In plants, with one exception, all the inclusions have been extranuclear. By contrast, some thirty virus diseases of animals are known in which specific inclusions are frequently found in the nuclei. The example in plants was found by Goldstein (1927) in a dahlia plant suffering from mosaic. It was an amorphous body of the X-body type, and as only one was seen can hardly be regarded as a characteristic feature of this disease. The absence of intranuclear inclusions in virus-diseased plants has afforded a striking difference between the virus diseases of animals and plants. In studying plants infected with severe etch virus, however, I have found that intranuclear inclusions are always present.

Although severe etch virus was first described by Johnson (1930) it has been little studied. In 1938 a culture of this virus was obtained by the courtesy of Dr W. M. Stanley. This virus causes in tobacco plants a severe chlorosis and greatly reduces the growth. Sometimes there is also great deformity, the leaves being reduced almost to the size of tendrils. In tobacco and all other solanaceous plants infected with this virus, two kinds of intracellular inclusion have been found. One is an amorphous, cytoplasmic inclusion similar to the X-bodies which have been described in many other virus diseases of plants. The other is an intranuclear inclusion and appears to be crystalline. The nuclei of plants infected with severe etch virus contain these plate-like inclusions with such constancy that they afford a valuable diagnostic character.

DISTRIBUTION AND PROPERTIES OF THE INCLUSIONS

Protein crystals have been described in the nuclei of a number of plant species believed to be healthy, especially in the Scrophulariaceae, but no members of the Solanaceae are mentioned in Tischler's list (1921) of plants containing intranuclear crystals. In the present work no crystals or crystalloids have been found in the nuclei of any healthy solanaceous plants or in those of plants infected with viruses other than severe etch.

Whether or not some animal viruses cause the production of intranuclear inclusions depends on the species of the infected animal and on the tissues infected. Some viruses cause their production in one type of cell but not in others, and other viruses may cause their production in one animal but not in another equally susceptible species. The age of the animal, the route of inoculation, and the virulence of the virus may all play parts in determining the formation of intranuclear inclusions. With the severe etch virus no such variation is obtained. All susceptible species of plants examined regularly produce the intranuclear inclusions. They are also present in every tissue of the infected plants, except at the growing points of roots and stems. In cells immediately below the growing points where cell division has ceased the inclusions can be found. It is possible that the great stunting of infected plants may be a result of this change in the nuclei.

The easiest method of detecting the intranuclear inclusions is examination of epidermal strips taken from below the veins on the undersurface of leaves showing pronounced chloroses. In such strips the nucleus of every cell may contain these abnormal inclusions. Sheffield (1936) found no inclusions in the guard cells of plants infected with several viruses including that of aucuba mosaic. Neither did she find any protoplasmic connexions between these and the other epidermal cells. She suggests that the virus moves from cell to cell along the plasmodesmata and suggests that the absence of inclusions in the guard cells is a result of the inability of the virus to enter them. In epidermal strips from severe etch plants the guard cells invariably contain intranuclear inclusions, but no cytoplasmic inclusions of the X-body type, which are common in other cells, have been seen (Pl. XXXIX, fig. 3). The intranuclear inclusions are found in all tissues more commonly than the X-bodies. Both types of inclusion occur in the following hosts infected with severe etch: *Nicotiana tabacum, N. sylvestris, N. glutinosa, Solanum*

lycopersicum, Datura Stramonium, Hyoscyamus niger. No inclusions have been seen in healthy plants of these species.

They appear to be produced equally readily in young and old plants. In the leaves, their production is restricted to those portions which show external symptoms. Leaves mature at the time the plant is inoculated rarely show symptoms: they contain little or no virus and no inclusions. On the other hand, if such mature leaves are rubbed with concentrated inoculum they become chlorotic, have a high virus content, and also contain the characteristic inclusions.

I have found that the severe etch virus is readily transmitted by *Myzus persicae* Sulz. and *M. circumflexus* Buckt. The relationships of this virus with its insect vectors seem to be similar to those described by Watson (1938) for *Hyoscyamus* virus 3 and *M. persicae.* The aphides soon cease to be infective after feeding on the source of the virus, and their efficiency as vectors, measured by the percentage of successful transmissions with single aphides, is increased by causing the insects to fast before feeding on the source of infection. Their efficiency also decreases the longer they are allowed to feed on the infected plants, more infections being obtained with insects which have fed for 2–5 min. than with those which have fed for 30 min. or longer. The intracellular inclusions, both intra- and extranuclear, are produced equally in plants infected by means of aphides and in those infected by rubbing.

The cytoplasmic inclusions are much more diffuse than the X-bodies produced by infecting *Solanum nodiflorum* with tomato aucuba mosaic virus (Henderson Smith, 1930). Most cells contain only one, but two have been seen on several occasions (Pl. XXXIX, fig. 1). They are definitely granular, appear to be aggregates, and their structure suggests that they have been built up by the aggregation of smaller particles as observed by Sheffield (1931) in aucuba mosaic. Sometimes, the X-bodies are almost spherical, when their diameters may vary between 6 and 25μ. More often, they are shapeless, elongated masses, when their length may be as much as 50μ.

By contrast, most nuclei contain more than one of the crystalloid type of inclusion, and as many as fifteen have been seen in one nucleus. All these are thin rectangular plates. Their size depends on the size of the nucleus and on the number of crystals present, the length of their sides varying from 3 to 10μ. When examined between crossed Nicol prisms they show no extinctions when viewed along any axis, but whether this is because they are really optically isotropic or because they are too small to give a visible effect cannot be stated.

The intranuclear inclusions usually appear about 12–16 days after infection, that is, about a week after external symptoms become obvious. The exact time depends on the size of the inoculated plants and on the environmental conditions. The X-bodies do not appear until a week after the plate-like inclusions are obvious in the nuclei. Both types of inclusion have been seen in the roots of diseased plants, but they appear here much later than in the leaves. Once formed, the inclusions persist for long periods, and they have been found in large numbers in plants as long as $4\frac{1}{2}$ months after infection.

Before the production of the crystals, the nuclei lose their regular outline, and their contents become more granular. The crystals appear to increase in size in a manner analogous to crystal growth in saturated solutions. The nucleoli are unaffected by the production of the intranuclear inclusions, and can be differentiated, apparently unchanged, in nuclei containing many crystals (Pl. XXXIX, fig. 4).

All microchemical tests have been made on epidermal strips as these contain most inclusions and are readily penetrated by the reagents. Both kinds of inclusion give Millon's and the xanthoproteic reaction for proteins. The nuclei of infected cells stained with Feulgen's reagent, but the amorphous inclusions and the intranuclear inclusions did not. With osmic acid the intranuclear inclusions stain brown, and with iodine in potassium iodide they stain yellowish brown. The crystals are unaffected by the usual methods of decalcification of fixed tissues.

Like the X-bodies produced by strains of tobacco mosaic virus (Sheffield, 1939) the amorphous inclusions disintegrate rapidly when pressure is applied to them. The intranuclear crystals are much more stable than either the cytoplasmic inclusions or the crystalline inclusions regularly found in plants infected with tobacco mosaic virus. When epidermal strips are mounted in water and teased with needles the crystals can be extracted from the cells (Pl. XXXIX, fig. 5). Such extracted crystals are insoluble in water, alcohol, ether, and chloroform. They dissolve in 1 % acetic acid, and when pressed with needles turn into amorphous masses. The refractive index is approximately 1·51.

The most suitable fixative for the intranuclear inclusions was found to be formol-saline (20 parts formalin, 80 parts 0·9 % sodium chloride solution). Fixatives containing acetic acid, picric acid or alcohol do not give good results as they coagulate the proteins. Also, the first dissolves the crystals. Flemming's solution without acetic acid gives good results. The crystals stain readily with acid dyes such as acid fuchsin and eosin. The simplest method for differentiating the intranuclear crystals is to mount an epidermal strip in an aqueous solution of eosin. The best

Fig. 1

Fig. 3.

Fig. 4.

Fig. 2.

Fig. 5

KASSANIS.—Intranuclear Inclusions in Virus Infected Plants (pp. 705 709)

method for staining fixed preparations is that of Kull, using acid fuchsin, toluidin and aurantia, which was used by Paillot (1926) in working with the polyhedral disease of silkworms. The crystals and the nucleoli stain red and the chromatin blue. With Heidenhain's haemotoxylin, the crystals stain black (Pl. XXXIX, fig. 2), and when placed in solutions of iron alum retain their colour longer than any other of the cell contents. In many ways these intranuclear inclusions accompanying infection with severe etch virus resemble the polyhedral inclusions found in infected silkworms. They are of a similar size, of the same stability, and react with stains in the same manner.

SUMMARY

Two kinds of intracellular inclusions in solanaceous plants infected with severe etch virus are described. One occurs in the cytoplasm and is similar to the X-bodies found in many other plant virus diseases. The other occurs only in the nuclei. These intranuclear inclusions appear to be crystalline, have the form of thin rectangular plates, and resemble the inclusions described in the polyhedral disease of silkworms more than any other type of previously recognized virus-inclusion.

I have much pleasure in expressing my gratitude to Mr F. C. Bawden for his help and encouragement.

REFERENCES

GOLDSTEIN, B. (1927). *Bull. Torrey bot. Cl.* **54**, 285.
JOHNSON, E. M. (1930). *Bull. Ky agric. Exp. Sta.* no. 306.
PAILLOT, A. (1926). *Ann. Inst. Pasteur*, **40**, 315.
SHEFFIELD, F. M. L. (1931). *Ann. appl. Biol.* **18**, 471–93.
—— (1936). *Ann. appl. Biol.* **23**, 506–8.
—— (1939). *Proc. roy. Soc.* B, **126**, 529–38.
SMITH, J. HENDERSON (1930). *Ann. appl. Biol.* **17**, 213–22.
TISCHLER, G. (1921). *Allgemeine Pflanzenkaryologie.*
WATSON, M. A. (1938). *Proc. roy. Soc.* B, **125**, 144–70.

EXPLANATION OF PLATE XXXIX

Fig. 1. Epidermal unstained living cells from tobacco infected with severe etch virus showing two granular X-bodies and a nucleus containing a number of crystalline plates. × 400.

Fig. 2. Cell similar to that in fig. 1 fixed in formol-saline and stained with haematoxylin showing five intranuclear inclusions. × 900.

Fig. 3. Guard cells fixed and stained as in fig. 2, showing the intranuclear crystals. × 900.

Fig. 4. Pith cells of stem fixed and stained, infected with severe etch virus showing crystalline plates in the nuclei. Nucleoli seem to be unaffected. × 400.

Fig. 5. Single crystal extracted from the nucleus. × 900.

(Received 27 April 1939)

THE MORPHOLOGY AND BIOLOGY OF THE BRAMBLE SHOOT-WEBBER, *NOTOCELIA UDDMANNIANA* L. (TORTRICIDAE)

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(With Plate XL and 10 Text-figures)

CONTENTS

INTRODUCTION

NOTOCELIA UDDMANNIANA L. is one of the most easily recognized tortricids, both in the larval and adult stages. The sluggish, dull brown larva has been found only on plants within the genus *Rubus*. On these it weaves a characteristic web, and this habit has provided the popular, descriptive name of the "Bramble Shoot-Webber" (Massee, 1937). The adult is readily identified by the large, rounded-triangular, reddish brown spot towards the lateral edge of the posterior margin of each forewing.

According to Meyrick (1927) *Notocelia uddmanniana* is a cosmopolitan species. Its range extends throughout Europe to Syria and North Persia,

also into North Africa. It has been recorded from all parts of the British Isles (Barrett, 1907; Blair, 1925; Carpenter, 1920).

Hitherto, no attempt has been made to discover in detail the habits of this tortricid. Literature on the subject is extremely scanty and consists either of short notes from collectors, stating the date and locality of adult captures (Burras, 1923), or from economic entomologists (Massee, 1937; Theobald, 1910) giving an account of the damage to cultivated Rubi. As far back as 1910 Theobald (1910) noted the damage caused by the larvae to loganberry canes. Since that date the acreage of cultivated varieties of *Rubus* has increased enormously and the moth has spread similarly. To-day, in certain fruit-growing districts of this country, it is regarded as a pest.

For the past two years the writer has undertaken a detailed study of the life history at East Malling. Observations have been made at all stages, both in the laboratory and under natural conditions.

One difficulty has been to give critical dates for any phase of the life cycle, as all stages are spread over a period of 3–4 weeks, e.g. the first adults emerge towards the end of June, but emergence is not complete until the third week in July. Nevertheless, it is hoped the results of this investigation will provide sufficient information to combat this tortricid in areas where serious damage is caused by the larva.

THE ADULT MOTH

Meyrick's (1927) description of the adult is as follows:

Forewings dilated, costa moderately arched; whitish-brownish, striated fuscous, costa posteriorly dark fuscous strigulated whitish; angulated edge of basal patch darker; central fascia fuscous, anteriorly indistinct, ending in a large rounded-triangular dark reddish fuscous whitish edged dorsal spot; an oblique fuscous fascia before apex, hardly reaching costa; extreme apex dark reddish fuscous. Hindwings grey.

Both sexes are similarly marked, though the female may have an olive green tinge in the forewings, making the general colours darker. The colour variations within the species are small and the size is fairly constant—the wing expanse of the male being 15–19 mm., that of the female 16–20 mm. (Pl. XL, fig. 1).

Earliest records for emergence during the past three years are: 21 June 1936, when one freshly emerged moth and another empty pupal case were found; 27 June 1937; and in 1938, the first evidence of adult moths was on 25 June, when one specimen was taken at night. Obser-

vations on the empty pupal cases were more satisfactory than searching for adults.

Text-fig. 8 shows how pupation is spread over one month, beginning at the end of May. Emergence of the adult covers a slightly shorter period, beginning during the fourth week in June. Thus it is possible, in the last few days of June, to find mature larvae, pupae and adults at the same time and in the same place.

During the daytime the moth remains inactive, usually resting on the underside of a leaf, but in woodlands near wild blackberries it may be found on plants other than the host. When disturbed it darts off with a quick but erratic flight, seldom travelling more than a few feet, and again alighting on a leaf or on the ground.

Light has a certain attraction for this species, and several specimens of the adult have been taken at night in lighted rooms. A more exact measure of the flight period is given from unpublished records of captures in the Rothamsted light trap (Williams, 1935) during the years 1933-5. Attached to this trap are a series of eight killing bottles which are each in use for one-eighth of the night—from half an hour after sunset to half an hour before sunrise—then automatically changed. At midsummer each bottle is exposed for approximately 45 min., increasing to 58 min. on 1 August. The total catch of *Notocelia uddmanniana* for each period during the three years was 1, 2, 15, 6, 2, 2, 0, 1. This shows that a few moths are active at almost any period of the night, but the maximum flight occurs in the two periods immediately preceding midnight. A study of the individual dates gives the earliest capture as 23 June in 1933, and the latest 23 July in 1935. Both these dates fall within the limits observed in more recent years at East Malling.

Under caged conditions the adult life varies from 10 to 19 days with an average of 14–15 days (Table III). Other moths which were sleeved on to the tips of young canes in the field—this being the nearest approach to natural conditions possible—lived from 9 to 15 days with 12 days as an average. No appreciable difference has been noted in the length of life of the two sexes, though it was not uncommon for caged females to outlive the males by 1 or 2 days.

Genitalia of the male

In the following description, the nomenclature used by Pierce (1914) has been followed.

· A distinguishing character of the genus *Notocelia* is the presence of two spines at the orifice of the aedeagus (Text-fig. 1). The uncus (*U*) is small, with many hairs at

the blunt apex. Each gnathos (*GN*) is covered, on the ventral surface, with long, slender hairs. The costal margin of each valva (*V*) is produced inwards, terminating freely in a point (*T*). The inner portion of each valva, or sacculus (*S*), is well covered with long hairs and short bristles; near the costal margin is a single, very large, blunt spine. Along the dorso-lateral edges of the valvae are numerous long hairs, changing to stouter bristles in the ventral half of the lateral margins. Many cornuti (*C*) are attached to the vesica; these appear to be easily shed, for in several preparations of the genitalia only the basal scars remained.
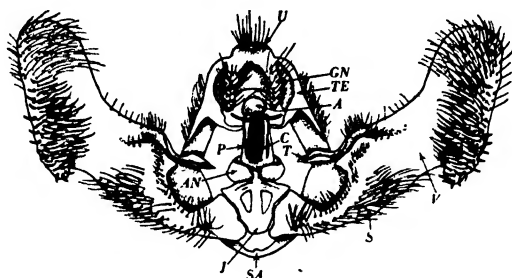
Text-fig. 1. Male genitalia. Ventral view (× 32). *A*, anus; *AN*, anellus; *C*, cornuti; *GN*, gnathos; *J*, juxta; *P*, penis; *S*, sacculus; *SA*, saccus; *T*, transtilla; *TE*, tegumen; *U*, uncus; *V*, valva.

THE EGG

Shortly after deposition the egg is translucent white, very soft, somewhat irregular in shape and enclosed in a thin but much pitted shell which remains soft until hatching. Colour changes are small, the white giving way to a pale cream which is retained until the larva emerges. The developing embryo only becomes obvious about 3 days before hatching, when the darkening head capsule may be seen through the fragile shell. Viewed from above (Pl. XL, fig. 2) the egg appears circular or oval; the dorsal surface is always convex, while the ventral surface, which is attached to the leaf by a glutinous substance, takes on the configuration of the leaf surface immediately beneath it.

Eggs are laid singly, either on the dorsal or ventral surface of a leaf near the growing point of a current year's cane. The very small, almost colourless egg is difficult to find in the field, and this was only overcome by releasing numbers of adult moths on an isolated plot of *Rubus*. It was then certain that after a few days eggs would be present on the plants, and by careful searching many were located.

When caged, the female shows no preference for either leaf surface. In the field, however, only 40 % of the eggs discovered were on the ventral

leaf surface. Those deposited on the dorsal leaf surface are very flat, and invariably found in the depression above one of the veins. On the underside of the leaf the egg is more spherical but much less regular in contour. These differences in shape arise because the soft-shelled and very liquid eggs are laid on two entirely different surfaces—above, the leaf is smooth and the egg can spread until it is almost flat; beneath, the egg is laid on a mat-like surface of leaf hairs and held by adjacent hairs. In fact the pressure of occasional leaf hairs make slight indentations in the lateral surface, hence the irregularity in outline. Table I illustrates this difference, giving the measurements of thirty eggs from each side of the leaf.

Table I. *Measurements of eggs of* Notocelia uddmanniana *from each leaf surface, in mm.*

Longest horizontal axis			Horizontal axis at right angles		
Max.	Min.	Av.	Max.	Min.	Av.
Dorsal surface					
0·68	0·56	0·64	0·56	0·44	0·48
Ventral surface					
0·60	0·44	0·53	0·52	0·32	0·41

Under field conditions never more than three eggs are found on a leaf; usually there is only one, and unless the infestation is severe very few eggs are laid on each new cane. This is confirmed by observations on the larval habit; it is unusual to find more than two or three first stage larvae feeding in the same terminal bud. Pl. XL, fig. 2, indicates the size, shape and position of the egg on the dorsal leaf surface. The numerous eggs on one leaf shown in the photograph were laid by caged females, and this is very different from the usual single egg per leaf deposited under natural conditions.

Oviposition takes place entirely by night, and eggs are never deposited until the second night after emergence of the adult from the pupa. The largest number of eggs is laid the first night; then for a period of 4–5 days the numbers are about equal, after which a gradual, though not constant diminution in the number occurs until death. Having laid all her eggs the female seldom survives for 24 hr. Table II shows the egg laying of three females which emerged on 21 June 1937.

Over 300 eggs may be deposited by a single female during her life of 10–19 days in the laboratory. In the field no moth lived longer than 15 days.

Tables III and IV illustrate the egg-laying capacities of this species. Field records were obtained by sleeving equal numbers of each sex on to

the growing tip of a young loganberry cane with a muslin bag, though an objection to this method was that some adults became entangled in the muslin folds where the bag was tied around the cane and died prematurely.

Table II. *No. of eggs deposited daily by 3 female* N. uddmanniana

Date	No. of eggs	Date	No. of eggs	Date	No. of eggs	Date	No. of eggs
28 June	—	2 July	109	6 July	50	10 July	8†
29	138	3	117	7	48	11	0
							(all dead)
30	118	4	78	8	39*		
1 July	105	5	58	9	3		

* One female died.   † Second female died.

Table III. *Eggs laid by* N. uddmanniana *when caged in the laboratory*, 1937

Date emerged from pupae	No. of females	Total no. of eggs deposited	Average no. of eggs deposited	Length of life of each female in days	Average length of life, days
17 June	3	869	290	13, 17, 19	16·3
21	3	871	290	12, 15, 15	14
24	5	1387	277	10, 12, 14, 14, 17	13·4
24	3	706	235	10, 12, 12	11·3
28	3	939	313	14, 17, 18	16·3
28	6	1549	258	13, 14, 14 15, 17, 17	15
28	3	622	207	14, 15, 15, 18	14·7
1 July	4	1052	263	12, 14, 15, 18	14·75
Totals	30	7995	266·5	452	14·5

Table IV. *Eggs laid by* N. uddmanniana *in muslin sleeves in the field*, 1937

Date emerged from pupae	No. of females	Total no. of eggs deposited	Average no. of eggs deposited	Length of life of each female in days	Average length of life, days
21 June	2	414	207	11, 14	12·5
23	3	787	262	12, 14, 15	13·7
25	3	741	247	10, 14, 14	12·7
25	3	644	215	9, 12, 12	11
28	3	694	231	10, 13, 15	12·7
28	4	648	162	6,* 8,* 11 14	9·75
1 July	3	738	246	12, 13, 15	13·3
1	2	421	210·5	11, 12	11·5
1	2	394	197	10, 13	11·5
Totals	25	5481	219·2	300	12

* These females died prematurely.

It will be seen from Tables III and IV that laboratory conditions apparently favour both egg laying and length of adult life.

Period of egg laying

Adults begin to emerge during the latter part of June, and within a few days, where the larvae have been noticed in numbers previously, sufficient empty pupal cases of both sexes may usually be found to indicate that mating is possible. Egg laying continues until the end of July.

Hatching

From a batch of eggs laid during any one night the greatest hatch occurs on the second day. A few larvae hatch the first day, 65–85 % hatch on the second day, and the remaining eggs all hatch by the following day, or the period may extend over 3 or 4 days.

The incubation period varies from 13 to 18 days, with an average of 15 days, in the field; in the laboratory the period is 8–11 days with slightly over 9 as the mean.

Emergence is through a longitudinal slit, cut by the larva, just above the lateral egg margin. The larva does not eat the shell, which remains attached to the leaf for many days after hatching.

THE LARVA

Feeding habits

On hatching, the young larva immediately migrates to the growing point of the cane on which the egg was laid. Here it crawls between the dorsal halves of a slightly opened leaf, and by means of silken strands webs the two halves together. Both first and second stage larvae are commonly found in this position. As the leaf develops, and becomes tougher, the larva moves up the cane to a more tender leaf, treating it in a similar manner. Before the hibernation stage is reached most of the larvae have ceased to inhabit a single leaf and burrowed into the actual growing point of a cane. At this period they are so small that no harm to the plant results from their feeding.

When hibernation takes place in August, the larva is on the current year's cane; on emerging in the following April it is on the fruiting cane of that year, since most of the cultivated Rubi produce canes which fruit in the second year and then die. Some larvae feed on the fruiting laterals of this cane, at first webbing together leaves, then when the blossom buds

appear they tunnel into the centres of the buds, eat the interior and leave only a shell composed of sepals and petals.

In April others leave the old canes on which they spent the winter, and wander on to the young canes, now a few inches above soil level. Here the halves of a young leaf are webbed together, or two overlapping leaves may form the initial larval habitat. As the cane grows and more leaves open, the web is enlarged to encircle these leaves, until finally the whole shoot-tip is tightly drawn together with the bud completely hidden (Pl. XL, fig. 3). Larvae still use the older leaves as a shelter, making excursions to the tender leaves and bud for food. Frass is often mixed with the silk strands forming the web, and sometimes an opening remains in one of the leaves through which it is ejected.

Those larvae on the fruiting laterals do not often remain after the blossom buds have reached an advanced stage and no further new leaves are being produced. Instead, they move on to the young canes, webbing together the leaves around the growing point as do the larvae which migrate immediately on emergence from hibernation. Except when the infestation is severe, not more than one larva is found in a web. The limiting factor to this solitary existence is reached when the larvae out-number the new canes. Then it is quite common to find as many as three or four living together in one web. Normally the larva lives in the same web on a new cane throughout its post-hibernation life, and finally pupates in the web.

Length of instars under laboratory conditions

In this experiment the larvae were reared in glass-topped tins, on a single, young loganberry leaf for the first and second instars; later the whole shoot tip was provided. Food was changed twice weekly, more frequently if necessary, and kept as fresh as possible by inserting the petiole or cut portion of the cane in a small heap of damp sand placed at the edge of the tin. Humidity was necessarily high, but no deleterious effect was noted; the temperature was fairly constant throughout the active phases of larval life, varying from 65 to 70° F., with occasional wider fluctuations. Sixty larvae were used each year, and the great advantage of this method proved to be the ease in handling the food when daily inspections were made for moulted head capsules. Also, should the capsule fall off the leaf, a clean, bottom surface of the tin made its discovery easy. Only one larva was kept in each tin.

From hatching to hibernating (Table V), under these conditions, is a

very short period; one larva commenced spinning its cocoon a fortnight after emergence from the egg.

Table V. *Duration of larval instars under laboratory*
conditions, in days

| | 1936–7 | | | 1937–8 | | |
Instar	Max.	Min.	Av.	Max.	Min.	Av.
I	10	7	8·5	10	5	7
II	8	4	5	8	4	5
III prior to hibernation	20	4	7	16	4	7·5
III after hibernation	10	6	7	10	6	7
IV	12	8	9·5	12	7	9
V	15	9	11	14	8	10

The larva normally hibernates in the third instar, but an occasional one was observed to spin its web in preparation for the quiescent, overwintering phase before passing through the second moult; in such cases the average duration of the second instar was prolonged, and ranged from 6 to 20 days, with an average of 12½ days. This was infrequent, for only 10 out of 120 hibernated before the third instar.

By removing the silken web forming the cocoon, it was found that these larvae had moulted again, within a short period of spinning up. Other specimens which did not moult twice before hibernation were left alone until they emerged in the following spring. In every instance a head capsule was found incorporated in the web, not loose, as would be expected had the larva moulted after beginning the resting period. This evidence suggests that moulting always occurred before the winter cocoon was completed, and within a few days of cessation of feeding.

The batch of sixty larvae placed in observation cages in the summer of 1937 produced two which went into the fourth instar before hibernating. One other pupated after moulting only four times, and a male moth emerged 5 weeks after the egg hatched. This was an exception, but such an occurrence was not confined entirely to the laboratory experiment, as will be seen when the instars are discussed under field conditions.

In the laboratory the second instar is the shortest. On an average the length of active life of the third instar is about the same before and after hibernation. Some larvae, however, feed for as long as 20 days prior to hibernating, never longer than half that period after emerging in the spring before moulting again. The third is the longest instar, but it is divided into two feeding phases, then in the fourth stage the larva lives

longer than either the first or second instars, and the final stage is a day
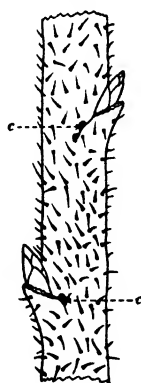or two longer than the fourth.

Field observations

Only approximate data can be given on the length of instar as it
varies considerably between different larvae. As this species prefers a
solitary existence in the spring, with only one larva in each cane tip to
avoid any confusion, the number under observation on potted plants
was limited to the number of young canes available.

Table VI. *Duration of larval instars in the field, in days*

Instar	1936–7			1937–8		
	Max.	Min.	Av.	Max.	Min.	Av.
I	20	13	16	20	12	15
II	13	8	10·5	13	8	10·5
III prior to hibernation	23	7	10	20	7	12
III after hibernation	21	14	16	19	12	14
IV	28	20	24	24	16	20
V	35	22	27	35	21	26

Egg laying begins at the end of June, and a fortnight later the first
larvae have hatched. Thus by the last week in July
many of the larvae are already in the second instar.
Emergence of the adult from the pupa is spread
over 3–4 weeks, hence some larvae will still be in
the first stage when the earliest ones, during the
second week of August, are already preparing to
hibernate. By the middle of August, a great many
have settled down in their winter cocoons, and
none of this generation has been found as late as
September at East Malling.

Upon cessation of feeding, the larvae leave the
tips of the canes and migrate towards the basal
half. The position chosen for the winter resting place
is always near a node (Text-fig. 2) commonly be-
tween the base of a petiole and the main cane. A
slight groove is excavated in the cane, and a tough
silken web spun around the outside; the entire in-
terior is covered with a finer silky lining. Any frass

Text-fig. 2. Drawing to
show position chosen
by larva of *N. udd-
manniana* to hiber-
nate. *c*, hibernation
cocoon.

voided during the preparation of this cocoon is found adhering to the
outer web. Sometimes the larva may find a sheltered position under a

piece of dead epidermis; this again is frequently near the base of a petiole. About 8 months are spent in hibernation.

Table VII and Text-fig. 3 show the period over which the larvae leave their hibernating cocoons and recommence feeding. In 1937 only a small number of larvae, on potted plants, were available for observation. The data for 1938 were obtained from a plot of mixed commercial varieties of *Rubus*, where numerous adults had been released the previous July.

Each lateral and new cane was inspected every other day and any larvae found were removed. The figures in Table VII represent the total number of larvae emerging from hibernation in the 48 hr. period immediately following the previous count.

Table VII. *Emergence of* N. uddmanniana *larvae from hibernation; counts made at 2-day intervals*

Date	1937	1938	Date	1937	1938	Date	1937	1938
26 Mar.	—	—	9 Apr.	—	25	23 Apr.	—	1
27	—	—	10	18	—	24	2	—
28	—	3	11	—	18	25	—	1
29	—	—	12	13	—	26	1	—
30	—	8	13	—	12	27	—	—
31	—	—	14	7	—	28	2	—
1 Apr.	—	13	15	—	7	29	—	—
2	—	—	16	6	—	30	1	—
3	—	25	17	—	3	1 May	—	—
4	1	—	18	5	—	2	1	—
5	—	30	19	—	2	3	—	—
6	8	—	20	2	—	4	—	—
7	—	29	21	—	1	5	—	—
8	20	—	22	2	—			

In 1937, 4 April was the first date when a larva was seen. Within 10 days, 75 % had left their hibernating quarters and the remaining 25 % came out over a longer period, the last being found on 2 May. By 18 April, 14 days later than the first larva appeared, 88 % had emerged, and 2 days later 90 % were feeding.

Warmer weather prevailed during the early spring of 1938, and it is possible that these favourable conditions were responsible for an earlier emergence from hibernation of the 1937–8 generation. The first record, 28 March, is a week earlier than that of the previous year. Again, Text-fig. 3 shows a rapid rise in the number emerging for the first 10 days, then an almost as rapid decline until only one or two fresh larvae appear at each inspection. This latter condition persisted for a fortnight in 1937, but only just over a week in 1938. A study of the percentage gives very similar results to the first year's readings. In 1938, 75 % of the larvae

are again feeding by 9 April, 12 days after the first emergence, and 90 %
4 days later.

Thus at East Malling over 90 % of the hibernating larvae of *N.
uddmanniana* are feeding early in the third week of April, after emerging
over a period of 16–18 days.

Emergence from the cocoon is affected by the larva biting a hole just
sufficiently large to allow its body passage to the outside.

In the field, the spring stages are considerably longer than in the
laboratory, no doubt due, in part at least, to the less favourable con-
ditions prevailing outside, in particular the lower temperatures.
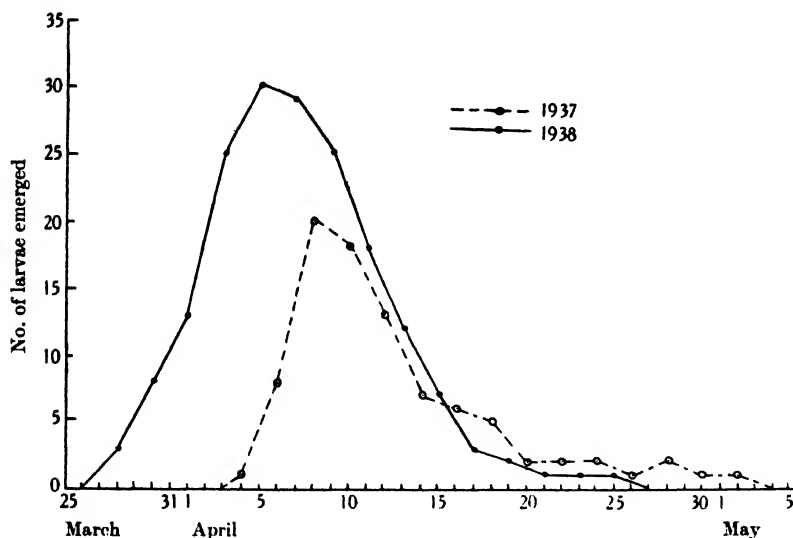
Text-fig. 3. Graphs to show emergence of larvae of *N. uddmanniana* from hibernation in
1937 and 1938, under field conditions.

Fourth stage larvae are present after the middle of April, and the
fifth and last instar early in May. Occasional fourth instar larvae are
found as late as the end of May and early in June, the result, most
probably, of a parasite present in the larva and retarding development.

In 1937, larvae of this species were discovered in very small numbers,
in the final instar, during the second week in August. These were brought
into the laboratory and fed; they pupated almost immediately, and on
24 August two female moths emerged, together with one male, while
another male appeared on 31 August. Mating took place and many
eggs were laid. The resulting larvae developed and, by the end of

September, all had hibernated; this time, with few exceptions, in the second instar. Only a few emerged in 1938, but those that did, matured, taking longer to complete the spring portion of the life cycle than the single generation from the adults of the previous June.

Whether the number of pupae of this early maturing group was sufficient to produce a partial second generation under natural conditions is doubtful. A large area of this district had to be searched in order to obtain the ten mature larvae from which only five adults emerged. A search was made to discover a partial, second brood in the field, but no evidence was obtained to suggest the presence of a second generation.

A similar phenomenon was noted with another species of Tortricid, *Cacoecia podana* Scop., but again only the mature larvae and an occasional pupa could be found. There was no trace of a second generation. The summer of 1937 provided exceptionally favourable weather conditions, which may account for this abnormally early maturity of certain larvae of these two species.

Morphology

First instar.

Shortly after hatching the larva is white, with a dark brown head, and approximately 1 mm. in length. The dorsal and dorso-lateral area of the prothorax, with the exception of the anterior edge, is covered by a light brown chitinized plate—the thoracic plate. Another smaller plate, similar in colour, is present on the posterior dorsal area of the anal segment; this is the anal plate. The thoracic legs are pale grey and very lightly chitinized.

By the third day a yellowish tinge has appeared in the body colour, and this increases in intensity, until, in the fully developed first stage, the larva is orange-brown. The larva measures 2 mm. in length at the end of the instar.

Head. The head capsule (Text-fig. 4 A, B) varies from 0·19 to 0·23 mm. in width, with an average of 0·215 mm. It consists of a V-shaped median dorsal frons (FR), confluent with the clypeus, separated on either side by an elongate, narrow adfrontal sclerite (ADF) from the epicranial plates (EP). Two groups, each of six ocelli, are situated laterally on the anterior half of the epicranium, one group on each side. The first four ocelli form a quadrant of a circle, the fifth posterior and ventral to number four, and the sixth is the most anterior, on a line midway between four and five.

The arrangement of the head setae is normal. The anterior setae $(A1, A2, A3)$ are so arranged that $A1$ is vertically above the third ocellus, $A2$, a much smaller seta, above the second ocellus and in a higher horizontal plane than $A1$, and $A3$ on the same level as $A1$, above the posterior edge of ocellus one. The anterior puncture (AP) is situated some distance behind $A1$. Behind or below the ocelli are three ocellar setae $(O1, O2, O3)$; $O1$ is just beneath the second ocellus, $O2$ posterior and ventral to the first ocellus, and $O3$ almost beneath $O2$, near the ventral edge of the head capsule. Three sub-ocellar setae $(SO1, SO2, SO3)$ are also present; $SO1$ in the anterior, ventral angle of the epicranium, a smaller seta, $SO2$, close to the anterior,

ventral edge of the fifth ocellus, and $SO3$ still more ventral, vertically between ocelli five and six. The posterior setae ($P1$, $P2$) and the posterior puncture (PP) lie in a straight line, diagonally across the epicranium; $P1$ is directly in line with AP and $A2$ near the adfrontal suture, $P2$ more lateral and posterior, with the puncture midway between them. There are two minute ultra-posterior setae (S) and two punctures behind $P2$. A single, small lateral seta ($L1$) is present a short distance behind $A3$.

On the adfrontal sclerite are two adfrontal setae, $AF1$ in a median position near $P1$, and $AF2$ more posterior. An adfrontal puncture (AFP) lies midway between the adfrontal setae.
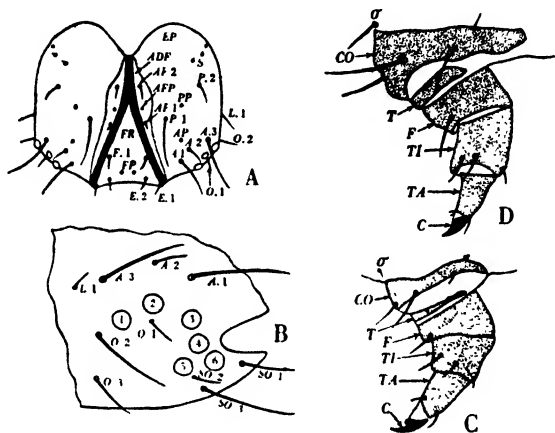
Text-fig. 4. A, head of first instar larva ($\times 125$). B, enlarged antero-lateral region of A. C, right mesothoracic leg of first instar larva, posterior view ($\times 250$). D, right mesothoracic leg of mature larva ($\times 32$). ADF, adfrons; EP, epicranium; FR, frons; $A1$, $A2$, $A3$, anterior setae; AP, anterior puncture; $AF1$, $AF2$, adfrontal setae; AFP, adfrontal puncture; $E1$, $E2$, epistomal setae; $F1$, frontal seta; FP, frontal puncture; $L1$, lateral seta; $O1$, $O2$, $O3$, ocellar setae; $P1$, $P2$, posterior setae; PP, posterior puncture; S, ultra-posterior setae; $SO1$, $SO2$, $SO3$, subocellar setae. The ocelli are numbered. C, claw; CO, coxa; F, femur; T, trochanter; TI, tibia; TA, tarsus.

Towards the anterior edge of the frons, near each adfrontal suture are the frontal setae ($F1$). Still more anterior and nearer the median line of the frons are a pair of frontal punctures (FP). Two pairs of epistomal setae ($E1$, $E2$) are present in the clypeal area, $E1$ in the extreme anterior angle adjacent to the adfrontal suture, $E2$ a little farther removed from the anterior margin, and in approximately the same vertical planes as the frontal setae.

Labrum (Text-fig. 5 C). This consists of two rounded lobes on a flat base, meeting anteriorly to form a shallow, median incision. There are three lateral setae on each side, a pair, one large and one small, near the lateral margin, the third very close to the antero-lateral edge. Three pairs of median setae are also present, a pair of small setae close to the median line, one-third of the length of the labrum from the median incision towards the base, a pair of much longer setae situated postero-laterally to the

median pair, and the third pair, short, antero-lateral, with their bases as far forward as the apex of the incision.

Mandibles (Text-fig. 5 B). The mandibles are alike, almost parallel-sided and heavily chitinized. From a postero-lateral position on the dorsal surface arises a long bristle; a small seta is found in a similar position on the ventral side of each mandible. Four teeth are present, the three outer ones pointed and largest, the fourth is blunt and considerably smaller. The inside, anterior edge of each mandible may sometimes be produced to form a small tooth, though more often it is flat.

Maxillae (Text-fig. 5 A). They consist of cardo and stipes (*S*), palpifer (*PF*), three jointed maxillary palp (*MP*), and a maxillary lobe (*ML*). Ventrally, two long, stout bristles are present on the stipes, one arising from a median position where the stipes broadens, the other near the antero-lateral edge. On the ventral, anterior margins towards the internal edge of both palpifer and the basal segment of the palp arises a stout bristle.
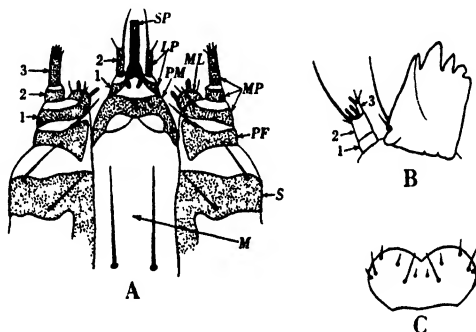
Text-fig. 5. Mouthparts of first instar larva (×250). A, maxillae and labium, ventral view. B, right mandible and antenna, ventral view. C, labrum, dorsal view. *LP*, labial palp; *M*, mentum; *ML*, maxillary lobe; *MP*, maxillary palp; *PF*, palpifer; *PM*, prementum; *S*, stipes; *SP*, spinneret.

On the outer side, the palpifer and two basal joints of the maxillary palp are equal in length, but ventrally the palpifer broadens rapidly towards the labium, whilst the basal segment of the palp narrows slightly. The terminal joint is three times as long as each of the other palpal segments, and bears at its apex two short spines, a pair of papillae each surmounted by a bristle, and another bristle on the interior edge.

Situated on the anterior margin of the basal segment of the palp, and adjacent to the labium, is the maxillary lobe. Near its apex are a pair of two jointed papillae, between them a stout spine, and laterally, on the side nearest the maxillary palp, another spine.

Labium (Text-fig. 5 A). Median, and on the ventral side of the head, is the labium, joined to the maxillae by a membrane. The mentum (*M*) is almost membranous, and bears two very long, thick bristles, which arise on the ventral side near the basal margin. A more strongly chitinized prementum (*PM*) carries a central spinneret (*SP*) and, laterally, labial palps (*LP*). Each labial palp consists of a short, broad, basal joint, and a long, thin, terminal segment, bearing at its apex a small

papilla from which arises a very long bristle, and a shorter bristle situated laterally and distal from the spinneret.

Antennae (Text-fig. 5 B). Each antenna consists of a narrow basal joint, a longer second segment, and a short, thin, terminal joint arising from the edge of segment 2 nearest the mandible. The basal and terminal segments are equal in length.

From the external, antero-lateral edge of the middle joint arises a long bristle, and near the base of this bristle is a much shorter one. Two elongate papillae, one dorsal and the other ventral, are situated at the apex of segment 2, together with an extremely minute bristle. The distal end of the apical segment bears a short bristle, a small papilla surmounted by a bristle, and a longer papilla.
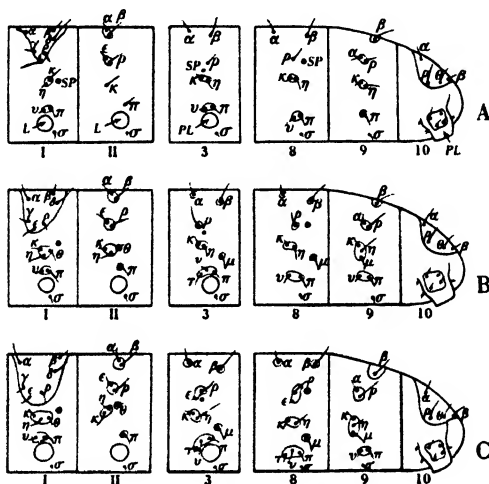
Text-fig. 6. Chaetotaxy of the larval instars. A, first instar. B, second instar. C, third instar. *L*, leg; *PL*, proleg; *SP*, spiracle. Roman numerals denote thoracic segments.

Chaetotaxy (Text-fig. 6 A). The method of designating the setae in these descriptions follows Fracker (1915). Six setae are present on the thoracic plate each side of the median dorsal line. Antero-laterally, almost on the edge of the chitinized area, is alpha, farther along the anterior lateral edge is gamma, with epsilon at the ventral extremity of the plate. Near the posterior edge of the thoracic plate are beta, slightly nearer the median line than alpha, delta, a long seta, situated at a lower level than alpha, and rho still more ventral, on a level about midway between gamma and epsilon. Beneath the ventral extremity of the thoracic plate and just anterior to the spiracle on the prothorax, two more setae, kappa and eta, arise from a common basal chitinized area. Still more ventral, a little above the leg, are nu and pi with a common base, and sigma is present on the ventral surface of the segment near the leg.

Both mesothorax and metathorax have identical setal maps. Alpha and beta arise from the same base on the dorsal surface, with alpha smaller and more dorsal than beta. Epsilon and rho have a common basal plate situated in a dorso-lateral position beneath alpha and beta. Kappa is a single seta beneath the rho group; pi

is ventral and posterior to kappa, above the insertion of the leg, and sigma is ventral, adjacent to the posterior edge of the leg.

The first eight abdominal segments are homologous in setal arrangement. Dorsally alpha and beta arise from separate tubercules, with alpha smaller, anterior and nearer the median line. Rho is large, situated near the dorsal edge of each spiracle, except in segment 8, where the spiracle is more dorsally situated, and here rho is directly anterior to the spiracle. A short distance immediately beneath each spiracle kappa and eta have a common base, with the exception, again, of segment 8, where the spiracle is farther removed from the setae and postero-dorsal to them. Very near the dorsal edges of the proleg bases on segments 3–6, and in homologous positions on the other segments, nu and pi arise from the same base, pi is the longer bristle and postero-dorsal to nu. Sigma is near the mid-ventral line, postero-ventral to the prolegs and in a similar position on the other segments.

On the ninth segment both beta setae arise from the same chitinized plate, occupying an almost median dorsal position. Alpha and rho are more anterior and lateral on a common base. Kappa and eta are together, beneath alpha and rho, eta slightly posterior and ventral to kappa. Still more ventral is pi, and sigma as on the other segments. In no first stage larva could nu be found on the ninth segment.

Four pairs of setae arise from the anal plate. Alpha and theta are on the same horizontal level with beta posterior to theta and nearer the mid-dorsal line. Rho is placed on the lateral edge in front of theta but not so anterior as alpha.

Each anal proleg has a small chitinized plate on the outside, containing three small setae and a puncture, arranged in the form of a square with the puncture in the posterior dorsal corner. There is a pair of setae in a median position on the posterior surface of each caudal proleg, another pair similarly placed on the anterior side, together with a pair on the same side just beneath the base of each proleg.

Spiracles. Nine pairs of spiracles are present in the first stage larva, one pair on the prothorax and a pair on each of the first eight abdominal segments.

Prolegs. Each proleg on the third to sixth abdominal segments possesses a uni-ordinal series of crochets varying from six to nine in number. Anal prolegs have five to seven crochets arranged uniordinally only along the anterior one-third of each margin.

Second instar.

The larva at this stage may be distinguished from the first instar by body colour alone—the orange tinge disappears rapidly after the first moult, and is replaced by brown. The head capsule may be shining black or dark brown; both thoracic and anal plates are darker and more heavily chitinized, though the anal plate is still paler than the thoracic one. Small chitinous areas surrounding the bases of the setae are darker brown. Orange red mandibles are conspicuous, and legs a darker grey. The head capsule varies from 0·29 to 0·39 mm. in width, with an average of 0·34 mm. When fully fed the larva measures 3–3·5 mm. in length.

Chaetotaxy (Text-fig. 6 B). Eta appears as an addition to the kappa tubercule on the mesothorax and metathorax. Each of the three thoracic segments has another seta, theta, added to the kappa group, arising from the same chitinous plate as kappa and eta on the prothorax, but on the other two segments it has a separate base, situated adjacent to the posterior margin of the kappa and eta tubercule.

Segments 1–6 of the abdomen contain tau as an addition, on the same basal plate as nu and pi, and anterior to these two setae. Nu is now present with pi on segment 9.

Dorsal and posterior to the tau group the first eight abdominal segments bear a new single seta, mu. This seta also appears for the first time on segment 9, where it arises from the same chitinous area as kappa and eta.

Prolegs. The crochets are still uniserial and uniordinal, but they are considerably larger than for the previous instar. Seven to thirteen crochets are present on each proleg except the caudal pair, where five to eight are arranged around the anterior edge only.

Third instar.

After the second moult the larva assumes the dark brown coloration which remains constant until pupation. In this stage the head capsule width of individual specimens varies from 0·46 to 0·64 mm., with an average of 0·54 mm. The head capsule is shining black, the thoracic plate more extensive than previously, either shining black or dark brown, with a paler anterior margin, and the anal plate slightly paler than the body. Around the bases of the setae the chitinous areas are more definite, as are the tubercules from which the actual setae arise. Thoracic legs which had become darker grey and more chitinized in the second instar are now almost black. The length of the larva at the end of the instar is about 6 mm.

Chaetotaxy (Text-fig. 6 C). The chitinous base from which rho arises on the first eight abdominal segments is produced anteriorly around the dorsal edge of each spiracle, and an extremely minute seta appears near the antero-ventral edge—this is epsilon. There was a trace of this new seta on the first abdominal segment in occasional specimens of the second instar larvae, but so few possessed it that it was doubtful whether epsilon should be generally present until this stage when all eight segments carry the seta. Tau is now present on segments 7 and 8. On the mesothorax and metathorax theta has moved farther away from the base of kappa and eta, and has a much enlarged basal plate.

Prolegs. Crochets are intermediate between uniordinal and biordinal, arranged as in previous instars with ten to seventeen crochets on each of the first four pairs, and seven to twelve along the anterior edges of the anal prolegs.

Fourth instar.

There is no appreciable difference in colour from that of the third instar larva. The head capsule increases in width to 0·77 mm., ranging from 0·68 to 0·86 mm., and the larva is about 1 cm. in length when fully grown at the end of the instar.

One conspicuous portion of the thorax is the thoracic plate, covering the entire dorsal area of that segment, and shining black, except the anterior margin, which is grey. The thoracic legs are black. No further additions to the setal arrangements occur after the second moult.

Prolegs. A definite tendency towards biordinal crochets, with some of intermediate length still present. Along the anterior margin of the anal prolegs from ten to nineteen crochets are present, on the other pairs the number varies from fourteen to twenty-eight.

Fifth instar.

In this, the final stage, the colour is at first similar to that of the preceding instar; on reaching maturity, however, the dark brown changes to a dull paler brown. Around the base of each seta or group of setae the chitinous plate is of a darker colour than the body. The thoracic plate is black with a grey-brown anterior margin, and the anal plate, though very dark, is more often brown than black. When fully fed the larva measures up to 1·5 cm. in length.

Head. This is shining black with setal arrangement very similar to the preceding instars. The width of the head capsule averages 1·23 mm., varying from 0·95 to 1·36 mm.
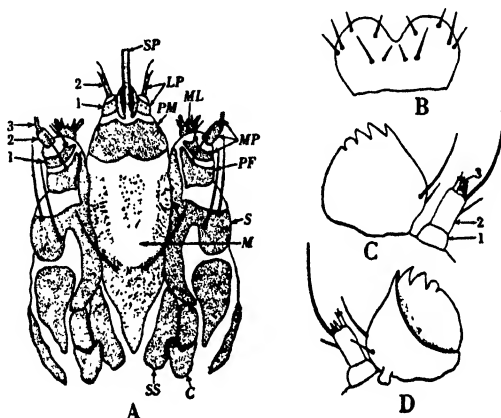
Text-fig. 7. Mouthparts of last instar larva (× 50). A, maxillae and labium, ventral view. B, labrum, dorsal view. C, right mandible and antenna, dorsal view. D, Right mandible and antenna, ventral view. *C*, cardo; *LP*, labial palp; *M*, mentum; *ML*, maxillary lobe; *MP*, maxillary palp; *PF*, palpifer; *PM*, prementum; *S*, stipes; *SP*, spinneret; *SS*, submental sclerite.

With the exception of differences in size and greater chitinization of all parts, the mouthparts of the mature larva resemble very closely those of the first instar larva.

Labrum (Text-fig. 7 B). Instead of a very acute apex to the incision midway along the anterior edge there is now a deeper and more rounded indentation. All the bristles are larger and approximate more closely to equality in size.

Mandibles (Text-fig. 7 C, D). These have lost their rectangular shape; they are now rounded laterally and relatively broader.

Maxillae (Text-fig. 7 A). Segment 2 of the maxillary palp (*MP*) has elongated until in the final instar larva it is much longer than either the basal or apical joints. The apical joint is considerably narrower in comparison with the other segments than in the first stage, and has three small papillae present on the apical extremity. From the terminal surface of the maxillary lobe (*ML*) arise two long slender papillae, another pair of papillae, two-jointed with the apical joint minute, a short central spine and from the side nearest the palp a larger bristle.

Labium (Text-fig. 7 A). The mentum is still lightly chitinized but more robust than in the early instars; the prementum is also larger.

Antennae (Text-fig. 7 C, D). A lengthening of the intermediate joint has occurred, and in the final stage larva it is more than twice as long as the basal joint. The apical joint is considerably reduced, as are the papilla and bristles carried at its extremity.

Chaetotaxy. No change in setal arrangement occurs after the third instar. It may be of interest to point out that individual larvae of the third, fourth and fifth stages seldom possess a complete set of setae. Occasionally a whole group may be missing from one segment, or only one seta from the basal plate. A seta is very often absent from the tau group on segments 1, 2 and 9 of the abdomen. If an entire group is absent there is no chitinous plate present to indicate the exact position, so this deficiency cannot be attributed to mechanical damage during the same instar or we should sometimes expect to find the base present.

Spiracles. Through each instar the relative size of individual spiracles remains constant. The prothoracic pair and those on the eighth abdominal segment are the largest and equal in size—about 0·08 mm. diameter in the mature larva. The others—on the first seven abdominal segments—are equal in size, but only a little more than half the width of the two larger pairs. A thick circular peritreme surrounds the opening of each spiracle.

Thoracic legs (Text-fig. 4 D). In all the instars the thoracic legs are identical in structure—the only difference is the degree of chitinization, each instar possessing a larger and stronger leg than the preceding one. The legs are conical, tapering from a broad coxa to the single claw. The *coxae* (*CO*) are incomplete on the outside. A large bristle is present in a median position on the inside, directly beneath sigma, and three bristles on both anterior and posterior sides, a small one at the dorsal edge towards the outer extremity, a larger bristle midway between the dorsal and ventral edges of the coxa, vertically above the lateral extremity of the trochanter, and the third on the same horizontal level but nearer the median bristle. The *trochanter* (*T*) is narrow, and the lateral extremities do not meet externally. No bristles are present. Each *femur* (*F*) is complete, with a pair of large bristles on the internal, ventral edge. *Tibiae* (*TI*) and *tarsi* (*TA*) are also entire, the former having three bristles on both anterior and posterior surfaces. These bristles become progressively larger from the outside inwards. Each tarsus has a pair of bristles near the external, ventral edge and a smaller pair on the corresponding internal edge.

Prolegs. A uniseries of biordinal crochets on the first four pairs varies in number from twenty-seven to forty-eight. Along the anterior margin of the anal prolegs are nineteen to thirty-four crochets, also biordinal. It is still possible to find the regular sequence of alternating large and small crochets broken by two of the same size.

Head capsule measurements in relation to Dyar's Law

Dyar (1890) devised a method whereby any inaccuracy in the observed number of larval moults could be detected. From studies on the larval instars of twenty-eight species of Lepidoptera, Dyar found that the width of head in successive instars followed a regular geometrical progression.

An analysis of the widths of moulted head capsules from *N. uddmanniana* L. over 2 years has given similar results to those obtained by Dyar (Tables VIII and IX).

Table VIII. *Measurements of the head capsules in all larval instars*
of N. uddmanniana, 1936–7

Instar	Max. width in mm.	Min. width in mm.	Av. width in mm.	Ratio	Calculated width in mm.
I	0·23	0·19	0·213	—	—
II	0·37	0·29	0·34	1·60	0·33
III	0·64	0·46	0·54	1·59	0·51
IV	0·81	0·68	0·75	1·39	0·80
V	1·36	0·95	1·24	1·65	1·25

Mean ratio = 1·56.

Table IX. *Measurements of head capsules in all larval instars of*
N. uddmanniana, 1937–8

Instar	Max. width in mm.	Min. width in mm.	Av. width in mm.	Ratio	Calculated width in mm.
I	0·23	0·20	0·216	—	—
II	0·39	0·31	0·34	1·57	0·33
III	0·61	0·46	0·53	1·56	0·51
IV	0·86	0·76	0·80	1·51	0·78
V	1·31	1·14	1·21	1·51	1·20

Mean ratio = 1·54.

The mean ratio is obtained by dividing the observed average width for each instar into that for the succeeding instar, and taking the average of the results.

Since the calculated widths agree so closely with those observed, it is most improbable that any moult has been missed. Moreover, the results for two successive generations approximate too closely to suggest any inaccuracy.

THE PUPA

Biology

For a period of about 3 days prior to pupation the larva does not feed. It becomes sluggish and the body colour changes to a paler brown. Slight lateral bulges appear on the meso- and metathorax, where the brown is replaced by a dirty white. These areas of paler coloration extend dorsally to the alpha-beta setae, laterally and ventrally. The head is partially retracted beneath the thoracic plate; the two posterior thoracic and the first two abdominal segments also contract. During this quiescent

period the alimentary canal is emptied and the frass excreted becomes attached to the outer surface of a small silken cocoon, which the larva spins round itself within the webbed mass of leaves. It is in this cocoon that pupation takes place.

Time of pupation.

In the field at East Malling, pupation has first been noted during the latter part of May—27. v. 36, 29. v. 37 and 20. v. 38 are earliest records—and continues for 4–5 weeks.

Table X. *Time of pupation of* N. uddmanniana *in* 1937

Date	No. of larvae	No. of pupae	Percentage pupated	Host plant
26 May	38	0	0·0	Loganberry
29	35	1	2·8	Wild blackberry
2 June	31	1	3·2	Loganberry
7	30	3	9·1	Loganberry
8	21	7	25	Wild blackberry
11	45	15	25	Wild blackberry
15	13	10	43	Loganberry
20	14	17	55	Wild blackberry
23	4	10	71	Wild blackberry
24	5	21	81	Wild blackberry
27	6*	25	89	Wild blackberry
2 July	2†	19	100	Wild blackberry

* Three larvae parasitized.   † Both larvae parasitized.

Immediately pupation began in 1937, bi-weekly collections of larvae and pupae were made either from wild blackberry or loganberry as in Table X. It has been considered that each of these counts was representative of the actual proportion of larvae and pupae present in this district on the dates recorded. Text-fig. 8 shows that only a few pupate during the first week; afterwards the number changing from larva to pupa increases, then remains fairly constant until pupation is almost complete. Towards the end of the period the rate of pupation again slows down. One exception is notable, that of 8 June, for which there is no obvious explanation.

In 1937, pupation began on 29 May, and was completed by the end of June.

*Duration of the pupal stage.*

In the laboratory the pupal stage lasted from 12 to 16 days, with an average of 13½ days. Observations in the field gave an average of 3 weeks for this phase, the shortest period was 17 days, and the longest 28 days.

Emergence from the cocoon.

Empty pupal cases are always found protruding from the silken pupal chamber in the web as far as the tips of the wing cases. No special device for rupturing the end of the cocoon has been observed. With the aid of the dorsal spines, a wriggling motion should be sufficient to force the pupa partly outside the cocoon.
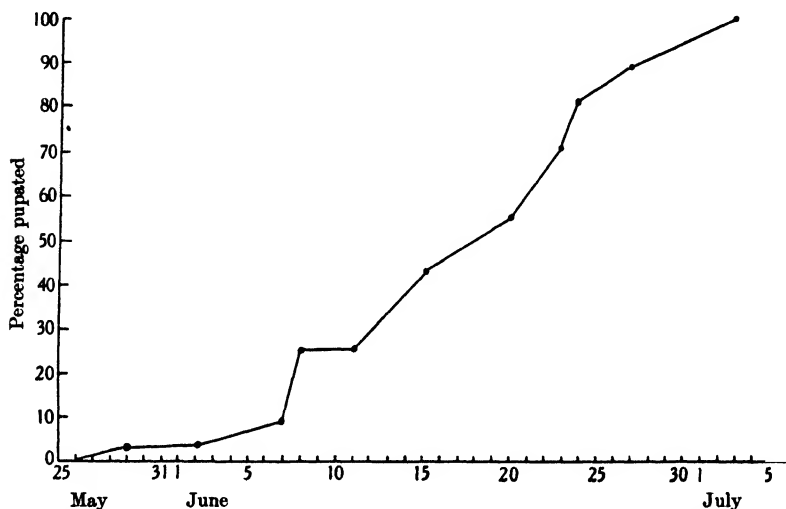
Text-fig. 8. Graph showing period and rate of pupation of *N. uddmanniana* in the field, 1937.

Emergence of the adult.

This occurs by a splitting of the pupal case along the mid-dorsal line (Text-fig. 9X) from the anterior edge of the prothorax to the posterior edge of the metathorax. Ventrally there is no fracture, but the antennae, together with the eyes, frons, labial palps and first coxae become free as one piece, and the mesothoracic legs tend to separate along the mid-ventral line.

Emergence occurs both by day and night in almost equal numbers. Frequent counts by day showed no preference for any particular period.

Morphology

The pupa is reddish brown, and always darker on the dorsal surface, the change of colour occurring rapidly along a narrow lateral area slightly dorsal to the spiracles. Individual specimens show a marked difference in colour intensity, varying from those

with almost black wing pads and dorsal area, and dark brown ventrally, to others which are chestnut brown above and orange brown on the ventral side.

With the exception of the metathorax, which is pitted and dull, the head and thorax and appendages of these two regions are glabrous. Ventrally the wings always extend to the fourth abdominal segment, and overlap about half its length (Text-fig. 9). Mid-ventrally the anterior pair of wings (W 1) do not meet, and here, posterior to the extremities of the mesothoracic legs ($L2$) may be seen a small area of the posterior pair of wings (W 2). Projecting beneath the wing tips, but not beyond them, are the metathoracic legs ($L3$). The antennae (AN) do not quite reach to the posterior extremities of the mesothoracic legs.
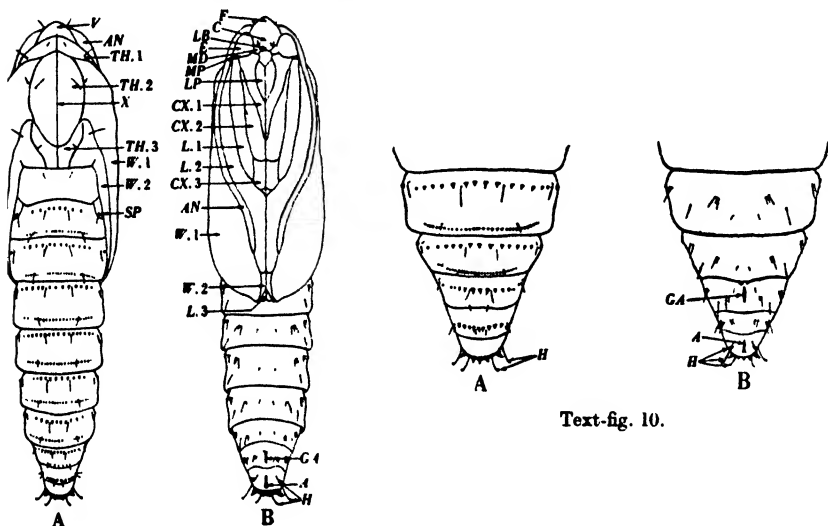
Text-fig. 9.

Text-fig. 10.

Text-fig. 9. Male pupa (× 8). A, dorsal view. B, ventral view. A, anus; AN, antenna; C, clypeus; CX, coxa; E, eye; F, frons; GA, genital aperture; H, hooks; L, leg; LB, labrum; LP, labial palp; MD, mandible; MP, maxillary palp; SP, spiracle; TH, thorax; V, vertex; W, wing; X, ridge along which pupa splits to allow emergence of adult.

Text-fig. 10. Female pupa. A, dorsal view. B, ventral view. A, anus; GA, genital aperture; H, hooks.

The abdomen has a minutely pitted surface, and is more dull than the thorax. In both sexes segments 2–7 possess, besides the normal setae, homologous to those on the mature larva, two transverse rows of backward-projecting spines on the dorsal surface. A row of large spines near the anterior margin reaches laterally to the Rho group of setae, and a row of considerably smaller spines, near the posterior margin of each segment, extends for the same distance. In both rows the spines decrease in size towards the lateral extremities. On segment 8 the anterior row is present in both sexes. The posterior row is seldom found on female pupae (Text-fig. 10), yet male

pupae nearly always have the two rows of spines, the posterior row somewhat shortened and not extending laterally beyond the beta setae. Only one row of spines is present on segment 9 in both sexes; these are of the larger size and situated transversely across the posterior half of the dorsal surface.

The distal end of the terminal segment is blunt and forms the cremaster. Four to six large spines project vertically from the dorsal edge. There are also four pairs of slender elongate bristles with recurved tips (H), a pair on either side of the anal pore and one hook on each side of the extreme lateral spines.

Segments 4–7 in the male and 3–6 in the female are movable. Functional spiracles (SP), visible externally, occur on segments 2–7 of the abdomen; those on segment 8 are vestigial. The male pupa (Text-fig. 9) possesses one genital pore (GA) on segment 9, the female a common opening of the bursa copulatrix and oviduct on segment 8. In both sexes the anal pore (A) is on the caudal margin of segment 10.

There is practically no difference in length between the two sexes. Measurements of fifty specimens of each sex gave an average length of 8·6 mm. for the male, with a variation from 7 mm. to 1·0 cm.; and the female pupa ranged from 6·3 to 9·8 mm. with an average of 8·6 mm. The female is, however, wider than the male.

<div align="center">PARASITES</div>

Two species of hymenopterous parasites and one dipterous parasite have been bred from the larva of *N. uddmanniana* L.

It is highly probable that the insect is kept in check to a considerable extent by the presence of an ichneumonid parasite, *Omorgus mutabilis* H.Gr. From several hundred larvae collected at East Malling during the past 3 years, the proportion found to be parasitized has not fallen below 36 % (Table XI).

<div align="center">

Table XI. *Percentage* Notocelia uddmanniana *L.*
parasitized by Omorgus mutabilis

Year	Total no. of larvae collected	No. of larvae parasitized	Percentage parasitized
1936	410	154	37·5
1937	343	123	36·0
1938	375	180	48·0

</div>

The adult parasite lays its egg in a young larva, probably the first stage. Bred female parasites, when caged with shoots containing first stage larvae, become excited, running over the leaves, and having located a larva, pierce the web and sometimes the leaf also with their ovipositor. That parasitism occurs before the spring feeding phase is certain, for larvae collected during hibernation and then isolated have several times produced parasitic cocoons.

The first indication that a larva is parasitized is partial cessation of feeding, sluggishness, and the skin becoming a darker brown and wrinkled. A few days later it becomes a grey-brown, and the parasitic larva may be observed to fill the entire body space. It breaks through the skin of its host and spins a grey, ovoid cocoon *in situ*.

The earliest parasitic cocoons are found by the middle of May, mostly from the fourth instar larvae. Other larvae of *Notocelia uddmanniana* L. reach the final instar, where the presence of the parasite may, in extreme cases, prolong the length of the fifth stage many days beyond the normal duration. After all healthy larvae have pupated, it is still possible to find occasional parasitized larvae for another week or 10 days. Occasionally the larva pupates before the parasite matures.

Omorgus mutabilis adults commence to emerge at the beginning of June and continue until the middle of July, or later.

This parasite is widespread in England. It has been bred from larvae of *Notocelia uddmanniana* received from Gloucestershire, Lincolnshire, Cambridge, Essex, Kent, Sussex and Devon.

The other larval hymenopterous parasite is an undescribed species of *Apanteles*. So far it has only been discovered in small numbers.

One specimen of a dipterous parasite (fam. Tachinidae), *Nemorilla notabilis* Mg., was bred. This parasite was undoubtedly present in the larva when collected, but did not appear until after pupation.

HOST PLANTS

Of those varieties of *Rubus* most extensively grown, only the loganberry and phenomenal berry suffer severely from attacks by this tortricid. Raspberry (*R. idaeus* L.) and the blackberries—Himalaya and Black Diamond (both probably selections from *R. procerus* P.J.M.) and Cut-Leaved (*R. laciniatus* Willd.)—are far less attractive as food plants, yet a few larvae are present on them each year. The numerous other species and hybrids of *Rubus* will all provide an adequate food supply for *Notocelia uddmanniana* L. to complete its life cycle.

Wild blackberry is definitely a favourite host plant, and is always a potential source of infestation when situated in the neighbourhood of newly planted plots of cultivated varieties of *Rubus*.

This tortricid has not been recorded from any plants belonging to a genus other than *Rubus*.

In England *Notocelia uddmanniana* L. is not at all uncommon on cultivated forms of *Rubus* in the main fruit-growing areas—Cambridge and Wisbech, south-east England and the Wye Valley. So far it is only in the first-named district that the larva of this moth causes serious damage, though sometimes it appears in sufficient numbers to cause anxiety to fruit-growers in the other localities. It must not, however, be overlooked that many of our present-day pests were at first noticed in small numbers, then increasing as the acreage of intensive cultivation of single crops increased.

No appreciable damage occurs from larval feeding prior to hibernation. It is from April until the larvae pupate that serious damage may be caused. The growing caterpillars feed voraciously—those on the fruiting laterals destroy the blossom buds, then move off to the young canes. Without exception, the terminal buds of webbed canes are destroyed and growth checked, to be continued later from the lateral buds, which normally would have remained dormant until the following year. The canes with much lateral growth are weaker than a single, stout cane; they also ripen less successfully in the autumn, and consequently there is a reduction in the yield of fruit the following year.

PRELIMINARY EXPERIMENTS ON CONTROL MEASURES

Apart from the tedious method of hand picking, no effective control has yet been devised. From a study of the life history it seems impracticable to apply control measures to the egg stage or young larvae because both coincide with the fruiting season. There are two other possibilities:

(*a*) Applying a winter wash to the canes to kill the hibernating larvae.

(*b*) Spraying or dusting after the larvae emerge from hibernation.

Canes containing hibernating larvae were dipped in a 10% tar distillate wash, but no control was obtained. It seems that the cocoon within which the larva spends the winter months is impervious to a tar wash.

In April 1937, when the first larvae began to emerge from hibernation, a spray containing 4 lb. of lead arsenate paste to 100 gal. of water was applied to a small plot of phenomenal berries. Plant growth was so rapid that new leaves with no spray residue were observed 2 days

after spraying. It was to these leaves that most of the larvae migrated. No difference between sprayed and control plants could be detected.

A similar experiment was carried out using 2 lb. of a proprietary derris extract and 6 lb. of soft soap per 100 gal. It was hoped that the derris on the older leaves would have a residual, contact, toxic action even if the larvae did not feed on the leaves. With one application when the first larva was seen and another 10 days later no control was obtained.

An atomized pyrethrum spray was used experimentally as a possible means of control in 1938. The spraying was done with a hand atomizer, using a pyrethrum extract diluted to 0·5 % pyrethrins content in a base of highly refined white oil of the kerosene type (viscosity 30° Saybolt at 100° F. and 99 % insoluble in concentrated sulphuric acid). On 4 April, before all the larvae had left their winter cocoons, twenty plants were sprayed. A count the following day showed that, out of forty-two larvae, forty were dead; one died later and the other survived. A field trial was immediately arranged to test this spray further. Owing to various difficulties it was not completed until 6 May. By this time all the larvae had spun large webs, and penetration by the spray was undoubtedly less than at an earlier date. The experimental spray, reduced to 0·25 % pyrethrins content, gave a control of only 6·5 %, and a proprietary spray[1] of unknown composition reduced the infestation by 3·7 %, each on plots of one-third of an acre.

The atomized pyrethrum is to be used again in 1939, when the application will be made at a much earlier date, before the larvae have had time to spin large webs.

SUMMARY

A detailed study of the morphology and biology of all stages in the life cycle of *Notocelia uddmanniana* L. has been made over a period of 2½ years.

This tortricid is a pest of loganberry in certain fruit-growing areas.

The widths of the head capsules in all larval instars have been measured and found to follow Dyar's Law.

In the East Malling district an ichneumonid, *Omorgus mutabilis* H.Gr., parasitizes 35–50 % of the larvae. A few specimens of an undescribed species of *Apanteles* have been bred from larvae, and also a tachinid fly, *Nemorilla notabilis* Mg.

Preliminary control measures have given poor results.

[1] The writer is indebted to Mr J. V. Lewis of Messrs Strawson's Ltd., who kindly loaned the atomizing unit and provided the proprietary spray.

The writer wishes to acknowledge his indebtedness to Dr A. M. Massee, who has supervised this work from the earliest stages, also to Mr J. E. Collin and Mr J. F. Perkins, who kindly identified the dipterous and hymenopterous parasites respectively, and to the Advisory Entomologists and fruit-growers who have helped with the collection of material. Also to Dr C. B. Williams who placed at the author's disposal his unpublished records of captures of *Notocelia uddmanniana* in the Rothamsted light trap.

REFERENCES

BARRETT, C. G. (1907). *The Lepidoptera of the British Islands,* **11**, 20. London.

BLAIR, K. G. (1925). The Lepidoptera of the Scilly Isles. *Entomologist,* **58**, 9.

BURRAS, A. E. (1923). An entomological visit to the Morvan (Central France). *Entomologist,* **56**, 139.

CARPENTER, G. H. (1920). Injurious insects and other animals observed in Ireland, 1916–18. *Econ. Proc. R. Dublin Soc.* **2**, 15, 267.

DYAR, H. G. (1890). The number of moults in lepidopterous larvae. *Psyche,* **5**, 420.

FRACKER, S. B. (1915). Classification of lepidopterous larvae. *Illinois biol. Monogr.* **2**, 1.

GILLIATT, F. C. (1932). Life history and habits of the three-lined leaf roller, *Pandemis limitata* Rob., in Nova Scotia. *Sci. Agric.* **12**, 506.

MASSEE, A. M. (1937). *The Pests of Fruits and Hops,* p. 146. London.

MEYRICK, E. (1927). *A Revised Handbook of British Lepidoptera,* p. 540. London.

PIERCE, F. N. (1914). *The Genitalia of the Geometridae.* Liverpool.

PIERCE, F. N. & METCALFE, J. W. (1922). *The Genitalia of the Tortricidae,* p. 66. Oundle.

THEOBALD, F. V. (1910). Report on Economic Zoology for year ending 30 Sept. 1910. *J. S.-E. agric. Coll. Wye,* **19**, 118.

WILKINSON, S. J. (1859). *The British Tortrices,* p. 69. London.

WILLIAMS, C. B. (1935). The times of activity of certain nocturnal insects, chiefly Lepidoptera, as indicated by a light-trap. *Trans. R. ent. Soc. Lond.* **83**, 523.

EXPLANATION OF PLATE XL

Fig. 1. *Notocelia uddmanniana* L. Female (× 2¼).

Fig. 2. Eggs of *N. uddmanniana* L. laid by caged adults (× 8).

Fig. 3. Damage to tip of cultivated blackberry cane by larva of *N. uddmanniana* L.

Fig. 3.

Fig. 1.

Fig. 2.

DICKER.—The Morphology and Biology of the Bramble Shoot-Webber, *Notocelia uddmanniana* L. (Tortricidae) (pp. 710–738)

FURTHER OBSERVATIONS ON *ANGUILLULINA DIPSACI* IN RHUBARB

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(With Plate XLI)

CONTENTS

INTRODUCTION

JOHNSON (1936) referred to the occurrence of *Anguillulina dipsaci* in dormant rhubarb root stocks in Yorkshire. It was stated that eelworm infection is associated with a diseased condition the symptoms of which are indistinguishable from those of crown rot described by Millard (1924) who reproduced the disease experimentally by inoculation with *Bacterium rhaponticum* isolated from diseased rhubarb. If it is accepted that in the field there are two separate diseases, namely bacterial crown rot and stem eelworm disease, observations made on material from a large number of holdings show that *A. dipsaci* infection is very widespread. A root stock showing typical disease symptoms similar to those described by Millard is likely to yield *A. dipsaci* in large numbers on laboratory examination. It is the almost invariable occurrence of the eelworm on rhubarb holdings in Yorkshire which cannot fail to suggest strongly that *A. dipsaci* is a most serious primary cause of disease in this host as it is in so many other cultivated plants. Reference has already been made (Johnson, 1936) to the similarity between stem eelworm disease in rhubarb and the same

disease in mangolds, as described by Goodey (1929). More recently Roebuck & Hull (1937) have described a crown canker of sugar beet associated with eelworm infection, and Walton (1936) has described a crown canker of parsnips attributed to *A. dipsaci.* White, in a series of notes (1933, 1934, 1935, 1936), reports on investigations on disease in rhubarb, in which he isolated a number of fungi and bacteria from diseased rhubarb and claimed to have isolated an organism comparable with *B. rhaponticum* as described by Millard. Negative results attended his attempts to reproduce the disease by inoculation. He concludes that "the negative results of this investigation, so far, are difficult to interpret on the view that the complex of symptoms usually termed crown rot is solely a disease of bacterial origin". White, like Millard, makes no mention of the presence or absence of *A. dipsaci* in his material obtained in the fields.

It is interesting to note that White refers to the occurrence of brown pockets in the centre of rhubarb roots, these pockets being "cut off by the formation of a skin" and being free from organisms. Millard also referred to pockets, enclosed by a skin, some of which he regards as lesions of bacterial crown rot, and he mentions the natural hollowing, remarking that it is found frequently in healthy plants, especially of the variety Victoria. The present writer has often seen these pockets, especially in the field when the roots are being split for propagation, and it is possible that the sterile brown areas described by White are comparable with these brown pockets which appear to be a prelude to natural hollowing. Plate XLI, fig. 1 shows a portion of a young sett raised from seed with a series of brown pockets in various stages of development, and in this case they would appear to be no more than a series of differentiating internodal hollows of the base of a flowering stem, which had not yet elongated. It is therefore evident that all rotting symptoms, or brown tissue, are not of necessity crown rot due either to bacterial or eelworm infection, and, in America, Beach (1923) has described a crown rot of rhubarb caused by *Phytophthora cactorum.*

The present writer's observations so far suggest that where active *A. dipsaci* infection is present in diseased rhubarb roots, there is not the tendency for the formation of a skin of suberized tissue completely to cut off the decayed lesion.

The incidence of *A. dipsaci* in association with disease of rhubarb is not confined to Yorkshire, for Mr W. E. H. Hodson, Advisory Entomologist in the Southern Advisory Province, has kindly informed the writer that he has recorded stem eelworm injury to rhubarb in his area. The

writer has also found *A. dipsaci* in diseased roots sent from Scotland and Middlesex. Johnson (1936) referred mainly to eelworm infection of dormant root stocks. The results of continued field observations and certain experiments on the bionomics of the eelworm are summarized in the present paper.

FIELD OBSERVATIONS

Continued opportunities of examining rhubarb plants from many holdings in Yorkshire confirmed previous observations, and strengthened the view that it is difficult to find a holding which is completely free from *A. dipsaci* infection of the rhubarb.

The ease with which infection can be carried in infected setts from field to field and from holding to holding is perhaps the chief source of trouble, and the importance of alternative cropping with the right type of crop, in conjunction with general hygienic measures, has been stressed (Johnson, 1936). Recently, cases have been encountered which serve to emphasize these points still further. On one farm, for example, the disease accompanied by very heavy eelworm infection was widespread in the three varieties, Victoria, Prince Albert and Dawes Champion. This state of affairs might be partly attributed to the grower's ignorance of the nature of the disease, and therefore to his failure to take proper steps in time to check it, but the rotation practised was rhubarb, potatoes, oats, seeds, and the capacity of the rhubarb strain of *A. dipsaci* readily to infect oats has been recorded by the writer. These preliminary observations have been abundantly confirmed in repeated experiments. On a second farm some six thousand roots in one field were badly diseased, heavy eelworm infection was present and the rotation had been rhubarb, oats, peas, rhubarb. Here again the alternative cropping might be criticized as likely to foster eelworm infection in the field. The farmer reported that the oats grown 2 years previously were badly "segged", and although this condition may have been due to frit-fly injury, it is not impossible that the oats were suffering from stem eelworm disease. Under experimental conditions the rhubarb strain of *A. dipsaci* has also been found to be capable of producing persistent and very injurious infection of peas.

Field inspections made during the growing period have furnished information on the extent to which the foliage may be infected by the eelworm, and diseased setts have also been taken from the field and grown in pots for observation during the summer. Millard, in his description of bacterial crown rot, states that the bases of the leaf stalks

may be swollen and that "such stalks show a brown discoloration at their junction with the crown". He also refers to the discoloration of the leaves and notes that the foliage of diseased plants often turns a characteristic puce colour. The present writer's observations have shown that rhubarb plants, suffering badly from crown rot associated with infection by *A. dipsaci*, may exhibit precisely similar symptoms in the foliage. On one of the holdings referred to earlier, where a field of rhubarb was very seriously affected by disease, large numbers of plants had reddish coloured foliage in May and on pulling at the stalks of such plants a portion of the diseased crown came away with the stalks. Laboratory examination revealed typical symptoms of both bacterial crown rot, as described by Millard, and stem eelworm disease, as noted by the writer. Thus, extensive rotting was present in the crown region extending often into the fang roots; brown discoloration sometimes, but not always, associated with swelling, occurred at the base of the leaf stalks and lesions extending into lateral buds from the diseased tissue below were observed. In all these lesions heavy infection by *A. dipsaci* occurred. These findings are similar to others noted in the field and in plants transplanted from the field into pots. The similarity therefore between Millard's description of bacterial crown rot and the symptoms of stem eelworm disease in rhubarb render it impossible to distinguish between the two diseases in the field. A disease in which *A. dipsaci* is involved is likely to be more difficult to control than one for which a bacterium alone is responsible, and it is essential, therefore, that the presence or absence of stem eelworm should always be determined in diseased rhubarb, for the result of such an examination will determine the steps to be taken to eradicate and prevent the disease. The question of the choice of suitable alternative crops is also much more important in relation to the stem eelworm, since certain crops may be potential carriers of the rhubarb strain, whereas this consideration would not be involved in the case of the bacterial disease.

EXPERIMENTAL

The purpose of the experiments to be described has been to induce eelworm infection of the rhubarb plant, to follow the course of the infection and accompanying symptoms, and to investigate the possibilities of transference of the rhubarb strain of *A. dipsaci* to other hosts, in particular to some of the crops commonly used in the rotation on rhubarb farms in Yorkshire. Experiments have been made both out of doors and in the cold glasshouse. In the indoor experiments plants were

grown directly from seed sown in pots containing eelworm free soil and the infection was introduced usually by the addition of dry infective plant material. Out of doors various methods have been used, namely ordinary pots sunk in the soil, glazed pots resting on the surface, and zinc cylinders sunk in the soil, as described by Hodson (1931). Infective material was added either before sowing the seeds of the host or after transplanted seedlings raised in eelworm free soil had become properly established. In all individual experiments each type of infection was carried out at least in duplicate with an adequate number of controls. Since infection experiments in ordinary pots are not always satisfactory, the cylinder method was used as a check on some of the pot experiments. In the following pages the experiments are summarized according to the host plant used.

Rhubarb

During the past 3 years a considerable number of experiments has been carried out and repeated using rhubarb as the host. In the first type of experiment rhubarb seed of the variety Victoria was sown in early spring in eelworm free soil to which in one series dried rhubarb chips had been added, the other being retained without infection as controls. Both ordinary pots and large glazed pots with a single outlet which could be corked when necessary were employed. The latter type of pot proved to be very satisfactory, possibly because the watering of the soil is more easily controlled than in the ordinary plant pots which may partially dry out quickly. The pots were kept in the cool glasshouse until late spring when they were moved outside. In the infected series, seedlings quickly became infected. Three weeks after sowing, for example, some seedlings exhibited distinct swellings of the hypocotyl below the cotyledons. Invasion of the cotyledons occurred, accompanied by distortion, and as the true leaves developed the infection passed into the petioles and leaves, resulting in very marked distortion of the foliage. A characteristic feature of eelworm infection of such leaves is a somewhat swollen corrugated appearance of both the petiole and infected veins. Infected leaves wilted, the base of the petioles turned brown and rotten, splitting occurred and the leaves eventually fell. A proportion of the seedlings died very early. As the season progressed all stages of what might be termed crown rot in miniature were observed in the young plants. Thus, in some, the top of the plant was easily detached and severe rotting, associated with heavy eelworm infection of the crown region, was revealed. Other plants exhibited earlier stages in the disease,

e.g. the plant shown in Pl. XLI, fig. 2, in which a cavity is just beginning to form beneath the crown. Heavy eelworm infection occurs in such plants all around the cavity and extends downwards into the tissue of the root, the extent of infection being perceptible in a sliced specimen by its light brown colour contrasting with the almost white healthy tissue outside it. All stages of the disease were obtained leading up to death of the seedling.

In a second type of experiment rhubarb seedlings were grown in cylinders sunk in the soil, the seedlings being transplanted into the cylinders in July. After the plants had fully recovered, dried infective rhubarb chips were mixed into the top soil of the infected series. Within 15 days infection was established in the foliage, several leaves were wilting, and some showed discoloured patches. These symptoms were associated with eelworm infection at the base of the leaf stalks. Subsequent examinations revealed the progress of infection which commenced in the leaf stalks and gradually passed up into the leaf itself by way of the main veins. Swelling of the base of the stalk sometimes, but not invariably, occurred, for in many cases the leaf stalk quickly rotted and split. Infected leaf stalks were often soft, and on being split open the interior was found to be discoloured and in many cases became dry and powdery; very large numbers of *A. dipsaci* in all stages could be recovered from these sites. Leaf infection was again characterized by the swollen, often discoloured and corrugated veins which internally revealed discoloration. Puckering of the leaf, especially at the junction of the main veins, frequently occurred. The eelworm infection had passed from the base of the leaf stalk to the ultimate tip of the leaf within 3 weeks after the introduction of infested material. Whole plants were lifted at intervals to ascertain the presence or absence of infection in the crown, but it was not until some 12 months after planting that there was any sign of disease in the setts. At this time it was clear that eelworm infection had invaded the root stock below the region of the lateral buds and rot extended from the outside of the plant to the tissue below the crown. It should be borne in mind that as the leaves and their stalks became heavily infected and wilted, they were removed and by this means a very considerable amount of potential infection was removed from the infected cylinders. All control plants remained free from infection.

During 1936 seedlings from the same batch as those used in the cylinder experiments were transplanted to a plot until all the foliage had died down, and in early November seven plants were transferred to

seven pots sunk in the soil. Three pots were infected by the addition of dry infective material; the other four were kept as controls and remained free from infection throughout the course of the experiment. In the infected pots the plants showed obvious signs of infection by mid-May the following year, the foliage developing typical symptoms; red spots frequently occurred on the leaves in association with infection. The plants were lifted on 5 December 1937, i.e. about 13 months after planting, and all showed signs of disease in the root stock. Two setts were not extensively diseased but well-defined lesions of brownish rot accompanied by eelworm infection occurred in the lateral aspect of each root and extended towards the crown. Certain lateral buds and the bases of fang roots near the main crown lesion were also affected. The third plant had reached a very advanced stage of the disease, the main crown having been destroyed and the very extensive rot had passed into some of the fang roots. The infection by *A. dipsaci* was extremely heavy. Pl. XLI, fig. 3, shows this plant cut longitudinally, and the symptoms recall those described and illustrated by Johnson (1936) of the disease in the field, developed as a result of natural infection. It is also clear that these experimentally produced symptoms are comparable with those typical of bacterial crown rot, as described by Millard. Eelworm infection has, however, not yet been observed in the leaves themselves in the field, but only in the bases of the leaf stalks. The reason may be that in most cases in the field the infection in the leaf stalk is preceded by well-established infection in the crown, and leaf stalk infection develops directly from that in the crown. In the infection experiments quoted, on the other hand, where young healthy seedlings are subjected to eelworm infection, the young leaf stalks become infected first and the infection advances rapidly in the leaves and finally invades the crown and root stock.

Oats

The preliminary observations that the rhubarb strain passed freely to oats in pot experiments using black winter oats have been confirmed. Pot and cylinder experiments using Victory oats also gave similar results. In the cylinder tests Victory oats sown in mid-March showed obvious signs of infection by mid-April. Typical severe "tulip-root" symptoms subsequently supervened in the infected series, whereas the control plants grew normally.

The fact that the rhubarb strain and an oat strain of *A. dipsaci* are reciprocally infective was reported by the writer, and this conclusion

has been confirmed in more recent experiments using infective oat material and rhubarb seedlings.

Peas

The pea plant was selected for trial because this crop is commonly grown in Yorkshire as one of the crops alternative to rhubarb, and on some farms where this crop is frequently sown the extent of rhubarb disease associated with eelworm infestation is very considerable.

One of the best growers in Yorkshire commonly makes use of the pea crop as one of the alternatives to rhubarb. In the past he has had no obvious signs of disease in his holding, but during the last 3 years, in spite of the fact that he did not introduce rhubarb stocks from outside, the disease has gradually gained ground, and is now sufficiently evident to cause serious concern. The writer has not, however, had opportunity to examine carefully pea crops on such farms for eelworm infection. Larval eelworms have been obtained from pea seedlings taken at random in the field but, although these eelworms possessed a buccal spine, they were not at a stage at which their identity could be established.

Controlled infection experiments were conducted first in pots in the cool glasshouse. Pea seedlings were raised in pots in soil to which eelworm-infested rhubarb material had previously been added, and a similar series were grown without the addition of eelworm infection. All the plants in the infected pots became heavily infested, and the infestation persisted throughout the life of the plants. The control plants remained healthy.

The experiment was repeated out of doors in cylinders and, as in the pot experiments, a persistent eelworm infestation occurred in the infected series associated with definite disease symptoms. Within 1 month after sowing in late March, all stages of the eelworm were recovered from the base of infected plants. Subsequent examinations revealed the passage of infection up the plants. The lower leaves were quickly invaded in both petiole and leaflet, and during the course of the season these lower leaves died prematurely. The basal portion of the stem eventually became discoloured and a similar brownish discoloration of the pith was associated with heavy eelworm infection. The control plants progressed normally and at the time of pod formation the surviving infected plants were never more than half the length of the controls. Some of the infected plants were so dwarfed that 3 months after sowing they had attained a height of only 3 in.

Mangolds

It was decided to test mangolds not only because this crop is grown on some rhubarb farms but also on account of the great similarity in the symptoms of eelworm disease in mangolds and crown rot of rhubarb. Two series of cylinder experiments were conducted; the first in April and the second in June. The results were similar in both cases. Fourteen days after sowing, the first signs of infection were noticed in a few of the seedlings in the infected cylinders. The early symptoms were the usual discoloured spots on the cotyledons continuous with a discoloured lesion extending along the petiole, and dissection of such parts disclosed *A. dipsaci* in numbers. One cotyledon only showed these symptoms. The progress of the infection produced distortion of the cotyledon, the infected side being shorter than the other, and twisting of the leaf finally occurred.

Pl. XLI, fig. 4, illustrates the kind of distortion which occurred. From this point the course of infection produced one of two effects on the plant. In some cases the seedlings failed to produce true leaves and such plants died, the hypocotyl region being heavily infected and severe rotting ensuing. Seedlings which produced true leaves showed some infection of the lowermost leaves, the petiole being invaded, resulting in some distortion of the leaf. The infection, however, failed to persist from this point onwards, and the surviving plants grew normally and no subsequent eelworm infection was observed.

Cabbage

It has already been stated that although the brassica crop is often the only crop alternative to rhubarb in the rotation, many growers are of opinion that a rest of only 1 year under a brassica crop is sufficient to effect a significant check on the disease. Examination of various brassicas taken in the field has so far yielded no signs of infection by *A. dipsaci*.

Infection experiments have been carried out in glazed pots in the cold greenhouse and out of doors, using cylinders. The results of these tests were similar to those obtained from the mangold experiments previously described. Control plants in both series remained free from infection, but seedlings in the infected pots and cylinders readily became infected. Thus, within 3 weeks after sowing in the cylinders, slight eelworm infection was observed just below soil surface. Twenty-six days after sowing, eelworm infection was apparent in the cotyledons in the

form of pale lesions in one cotyledon continuous with a lesion in the petiole. Distortion of infected cotyledons occurred as with mangolds, and growth of the petiole was frequently very much reduced. In some cases the stems of the infected plants were swollen, giving a corrugated appearance, and in some there was a distinct bulbous swelling at the top of the stem. All stages of the eelworm were recovered from infected parts. Later examinations showed that although in a few cases infection occurred in the base of true leaf stalks, the infection quickly died out and it seemed safe to assume that under the conditions of the experiment the rhubarb strain of *A. dipsaci* is unable to produce a persistent infection in cabbage.

Weeds

During the course of these experiments the opportunity was taken of examining for eelworm infection adventitious weeds which appeared in the pots and cylinders. Chickweed (*Stellaria media*) occurred freely and regular examinations made throughout the growing season proved that the rhubarb strain of *A. dipsaci* transfers freely to chickweed. In groundsel (*Senecio vulgaris*) and plantain (*Plantago lanceolata*), the latter being transplanted into infected cylinders and pots, no infection could be detected. A few *Rumex* seedlings, which were probably *R. crispus* and *R. acetosella*, became infected, but so far it is not known to what extent the rhubarb strain of *A. dipsaci* is capable of maintaining itself permanently on these weeds.

It is clear that the strain of *A. dipsaci* occurring in rhubarb is a generalized type in its feeding habits, which emphasizes the need for precise information as to what cultivated hosts are likely to carry infection in order that the grower may make a suitable choice of crops to use as alternatives to rhubarb in the rotation.

SUMMARY

Continued field observations on disease in rhubarb accompanied by infection by *Anguillulina dipsaci* are recorded. The symptoms, both in the foliage and in the root stock, are similar to those described for bacterial crown rot by Millard.

In infection experiments using the rhubarb strain of *A. dipsaci* symptoms of disease similar to those found in the field were reproduced in rhubarb seedlings and setts. The course of eelworm infection in the young leaves and in the crown in these experiments is recorded.

Fig. 1.

Fig. 2.

Fig. 3

Fig. 4.

Host transference experiments have shown that the rhubarb strain of *A. dipsaci* is not rigidly restricted in its feeding habits for it is capable of producing persistent infection in oats, pea and chickweed. It is also capable of producing only temporary infection in cabbage and mangold. Infection is also recorded in *Rumex* spp. but insufficient evidence is available as to its persistence on these hosts.

Incidence of *A. dipsaci* is recorded in rhubarb from Scotland and southern England in addition to Yorkshire.

The writer desires to thank his colleague Mr C. E. Hudson for his continued interest and assistance, and Mr J. Manby and Mr W. R. Wilson for the photographs.

REFERENCES

BEACH, W. S. (1923). A crown rot of rhubarb caused by *Phytophthora cactorum*. Abst. in *Rev. appl. Mycol.* 2, 433.

GOODEY, T. (1929). The stem eelworm, *Tylenchus dipsaci* (Kühn, 1858). Observations on its attacks on potatoes and mangolds with a host-list of plants parasitized by it. *J. Helminth.* 7, 183.

HODSON, W. E. H. (1931). The stem and bulb eelworm, *Tylenchus dipsaci* (Kühn) Bastian. A further contribution to our knowledge of the biologic strains of the nematode. *Ann. appl. Biol.* 18, 83.

JOHNSON, L. R. (1936). Observations on the occurrence of *Anguillulina dipsaci* (Kühn, 1858) on rhubarb in Yorkshire. *J. Helminth.* 14, 77.

MILLARD, W. A. (1924). Crown rot of rhubarb. *Bull. Univ. Leeds and Yorks. Coun. Agric. Educ.* no. 134.

ROEBUCK, A. & HULL, R. (1937). The stem eelworm (*Anguillulina dipsaci*). Attacks on sugar beet. *J. Helminth.* 15, 243.

WALTON, C. L. (1936). *Anguillulina dipsaci* (Kühn) as a cause of parsnip canker. *Rep. agric. hort. Res. Sta. Bristol*, p. 156.

WHITE, H. L. (1933). Rhubarb disease. *Rep. exp. Res. Sta. Cheshunt*, p. 54.

—— (1934). Vegetable diseases. *Rep. exp. Res. Sta. Cheshunt*, p. 46.

—— (1935). Crown rot of rhubarb. *Rep. exp. Res. Sta. Cheshunt*, p. 43.

—— (1936). Rhubarb crown rot and allied diseases. *Rep. exp. Res. Sta. Cheshunt*, pp. 54-5.

EXPLANATION OF PLATE XLI

Fig. 1. Crown of rhubarb plant sliced to show non-pathological internodal hollowings developing in base of flowering stem.

Fig. 2. Rhubarb seedling sliced to show formation of cavity below crown resulting from *A. dipsaci* infection.

Fig. 3. Rhubarb sett grown in eelworm infected soil showing advanced symptoms of crown rot accompanied by heavy eelworm infection.

Fig. 4. Mangold seedlings infected by the rhubarb strain of *A. dipsaci*. Note distortion of cotyledon.

THE EFFECT OF CLIMATIC EXPOSURE ON TEXTILE FIBRES AND FABRICS

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(With Plate XLII and 3 Text-figures)

CONTENTS

1. INTRODUCTION

PRIOR to 1930 the relevant technical literature contained occasional references to observations made on the "mildewing" of textile fabrics, and several writers on the subject expressed views both on the causes of "mildewing" and the treatment to which fabrics should be subjected

for protection against it. Such views, however, were seldom well supported by experimental evidence. A review of this literature was given by Thaysen & Bunker in 1930.

Because of the lack of well-authenticated information and also because of the practical importance of the subject, the writers undertook, with the approval of the Department of Scientific and Industrial Research, a comprehensive investigation of the subject. It was decided to expose to a range of climatic conditions samples of fabrics of a wide and varied nature so that evidence might be obtained regarding both the participation of micro-organisms in the "mildewing" of fabrics, and the conditions under which "mildewing" and other deterioration of fabrics would occur. Subsidiary objects were to ascertain the types of organisms which are destructive to textiles, a characterization of the nature of the damage caused, and an elaboration of methods by which the damage could be avoided or minimized.

2. METHODS

For this purpose a range of fabrics was exposed at one or more of the following stations, which were selected with a view to including as wide a range of climatic conditions in the test as possible.

> Australia (Darwin), (Broken Hill South), (Adelaide)
> Ceylon (Peradeniya)
> Cyprus (Nicosia)
> England (Holton Heath, Dorset)
> Federated Malay States (Kuala Lumpur)
> Kenya (Nairobi)
> South Africa (Pretoria)
> Trinidad (St Augustine)
> Ceylon (Colombo Harbour, for marine exposure)

The materials exposed comprised three types of cotton fabric, in each case with its corresponding yarn sample; two types of linen canvas; one wool fabric; one hemp fabric; two silk fabrics, with and without gum discharged; three rayon fabrics; and one jute fabric. The rayon fabrics had been prepared, in one case by the cuprammonium process, in another by the viscose process; the third type was a cellulose acetate fabric. Detailed analyses were on record of all the various fabrics, and it was possible, therefore, to estimate to what extent a change in the fabric during exposure might have been influenced by peculiarities in the fabric itself.

Through the collaboration of scientific workers at the stations mentioned it was possible to expose the fabrics under all conditions likely to be met with during the normal use of fabrics. The actual procedure for the exposure was communicated to the supervisors with the samples of fabrics sent to them from the writers' laboratory. Prior to dispatch the fabric samples, measuring 10×7 in., had been placed in stoppered glass tubes and subjected to a careful process of heat sterilization in order to

eliminate all harmful micro-organisms which might have been contained in the fabrics. Suitable aluminium frames for holding the fabrics during exposure were also supplied.

The following methods of exposure were adopted at all stations (except at Colombo Harbour, where the fabrics, suspended in their frames, were immersed in the sea at the guide pier to the harbour slip-way, 3 ft. below the surface and at such distances apart that they were unable to foul each other).

Two frames of each fabric (except of hemp, of which only one was taken) were used for each series of experiments. In one series the samples were buried vertically in open, not grass-covered, soil. In another series two frames of each fabric were hung in a place protected from the direct rays of the sun, but not sheltered from rain, and in a third series two frames of each fabric were hung in direct sunlight.

The various samples were examined periodically during exposure and the experiments were continued for a period not exceeding one year. Where the periodical examination revealed that some of the samples had deteriorated markedly before the year had elapsed they were removed at once, irrespective of time of exposure. In removing the samples handling was avoided as far as possible. In the case of the samples which had been buried, any loose soil adhering to the fabric was removed by gentle shaking and then the sample was immediately placed in its proper tube without further treatment. The whole operation was carried out in a sheltered place. In the case of samples which had been badly damaged, care was taken to avoid tearing. Any samples which had deteriorated more rapidly than others, and had in consequence to be returned to their tubes earlier, were kept in as cool a place as possible so that their further deterioration would proceed slowly, if at all.

As it was thought desirable to compare the microflora of the soils to which the fabrics had been exposed with that which might be found on the fabrics after exposure, provision was made for soil samples to be forwarded in suitable containers to the writers' laboratory together with the exposed fabrics.

The samples exposed in the sea at Colombo Harbour were examined at least once a fortnight during exposure and, as soon as serious rotting was observed, they were returned to their respective tubes and dispatched to England.

At the home station, in Dorset, a sufficient number of fabric samples over and above the standard number was exposed to make it possible to carry out analyses at frequent intervals.

At practically all the stations abroad the rate at which the fabrics were destroyed in the soil was much greater than had been expected and, not infrequently, most of the fabrics had disappeared when the samples were removed for final examination. For this reason further samples were sent to the stations in the Malay States and in Ceylon. On return to the laboratory the various colonial samples were stored on ice until they could be examined. The home samples were analysed immediately on their removal from the exposure ground.

Since the purpose of the investigation was to ascertain the part played by micro-organisms in damage suffered by the exposed fabrics it was desirable to use as many different culture media and as varied a technique as possible in order to isolate all the organisms which could be made to grow artificially.

. In addition to the ordinary bacteriological media, therefore, such as broth agar, broth gelatine and wort agar, various others containing cellulose and hemicelluloses

as carbohydrates were employed in the process of analysis. The cellulose medium contained the following additional substances:

Dipotassium hydrogen phosphate 0·1 %
Magnesium sulphate (crystalline) 0·1 %
Sodium chloride 0·1 %
Peptone 0·2 %
Calcium carbonate 2·0 %
Tap water 97·5 %

In the analyses both aerobic and anaerobic mesophilic and thermophilic organisms were searched for and series of inoculated media were incubated both at 30 and 65° C., in each case in the presence and the absence of air.

For the detection of wool-decomposing micro-organisms a suitable part of the exposed wool fabric was placed in containers with a piece of sterile wool and a convenient quantity of food solution of the composition referred to above, as used for the isolation of cellulose-decomposing organisms.

3. RÉSUMÉ OF EXPERIMENTAL OBSERVATIONS

The brief account given above of the plan of the investigation and of the technique adopted will have made it clear that the examination of the various exposed fabrics involved a very large number of bacteriological analyses of which it would be impossible to give a detailed account in these pages. In this communication, however, a résumé will be given of the observations made in so far as they have a bearing on the problem of the possible participation of micro-organisms in the deterioration of fabrics exposed to climatic conditions. For the purpose in view these conditions have been divided into five different groups. The first comprises those conditions which cause the complete and continuous saturation of the fabric with water. Such conditions were represented in the tests by the exposure trials in Colombo Harbour. The second represents the exposure to permanently damp, but not waterlogged conditions, such as are met with in most soil exposures and in shade exposures at certain tropical stations. In the shade exposure trials, the test fabrics were hung in the air in their respective frames and were shielded from all direct sunlight, but not from rain. Though it was assumed that these two sets of conditions would be similar as regards moisture conditions it was appreciated that the similarity did not take into account the important influence which might be exercised by differences in microflora in the two habitats.

The third condition involved exposure of the fabrics to occasional wetting with subsequent drying, as represented by shade exposure of the fabrics in temperate climates; the fourth to shade exposure in arid

climates where the moisture content of the exposed fabrics would be permanently below the minimum at which micro-organisms could be active. Finally, the fifth group comprised exposure of the test fabrics in the sun, some at high humidities, as in tropical climates; some in the sun in temperate climates, and others in the sun in arid climates. This group was included for the specific purpose of studying the influence of sunlight on exposed fabrics.

4. The part played by micro-organisms in the damage suffered by textiles on climatic exposure

(a) *Exposure of fabrics to waterlogged aerobic conditions*

In the test three types of cotton fabric were exposed with two samples of linen canvas and one each of hemp and wool. One striking feature of the exposure was the unexpectedly rapid deterioration of the materials. The officer supervising the exposure, which was carried out in Colombo Harbour, remarked on this point in his report. His first examination of the various fabrics was made 15 days after their immersion, when it was found that two of them, the Egyptian cotton and the wool, had partly broken away from the frames supporting them. Subsequent daily examinations indicated a rapidly progressing destruction, so rapid in fact that a fabric (American cotton) which on one day appeared sound, on the following day had collapsed in its frame. Only in the case of one of the flax canvases, a very thick and closely woven fabric, was it necessary to continue the exposure for 50 days before a complete destruction of this material had occurred; in most cases the fabrics were destroyed in less than 30 days.

Compared with the various cellulosic fabrics the woollen material decayed particularly rapidly in spite of being considerably heavier than the most closely woven cotton fabric. This observation does not agree with that of Dorée (1920) who found wool more resistant than cotton when exposed in sea water in a temperate climate.

In analysing the results obtained it must be admitted at once that the expected direct èvidence of the participation of micro-organisms in the deterioration of fabrics under waterlogged conditions could not be secured from the bacteriological analyses of the exposed samples. The anticipated presence of overwhelming numbers of cellulose-decomposing micro-organisms on the cellulose fabrics and of wool-decomposing types on the wool was greatly obscured by the presence on the damaged fabrics not only of a varied secondary microflora which it is exceedingly

improbable could have taken a direct part in the deterioration of the fabrics, but also of types expected to be exclusive to cellulose fabrics on the wool sample, and of wool-decomposing types on the exposed cellulosic fabrics.

It was necessary, therefore, to have recourse to a more indirect method for settling the nature of the agency which had caused the deterioration. For this purpose the microscopic methods devised by Fleming & Thaysen (1920) and by Bright (1926) for cellulose fibres were the only methods available. The claim made for these tests that it is possible by them to distinguish microscopically between microbiological damage on the one hand and chemical, physical and mechanical on the other, has been confirmed for cellulosic fibres by Searle (1924) and Burns (1927). By these microscopical examinations no difficulty was found in ascertaining that the three cotton fabrics had deteriorated extensively owing to attack by micro-organisms. In all the cases examined the swelling test of Fleming & Thaysen and the Congo red test of Bright showed that practically every hair in the deteriorated fabrics had the appearance under the microscope which is characteristic of microbiological destruction. In no single instance was action of a non-biological agency detected.

، On the evidence of the microscopic tests the conclusion was drawn, therefore, that the destruction of all the cellulose fabrics exposed in the sea at Colombo under waterlogged conditions was due solely to microbiological activity, thus confirming Dorée's (1920) laboratory tests by large-scale exposure trials.

In the case of the exposed wool sample a microscopic examination was much less conclusive. Here the expected accumulation of epithelial scales was missing. Such accumulation might have indicated activity by proteolytic micro-organisms. In its stead was found an aggregation of fragments of wool hairs of a much smaller diameter than that of normal hairs, an appearance which indicated the elimination of the scales and a subsequent exposure of the core of the hairs. There is, at present, no evidence to confirm that such changes in wool are characteristic of microbiological activity, and since the evidence obtainable through bacteriological analyses also failed to yield conclusive evidence it is impossible at present, and from the exposure tests in Colombo Harbour, to arrive at a definite conclusion as to the agency responsible for the destruction of the immersed wool fabric.

Though the direct bacteriological analyses of the various sea-exposed fabrics failed to give an unequivocal answer to the question of the nature

of the agency responsible for the decay, they were not without interest in other respects. Thus they confirmed a widely held view (Thaysen & Bunker, 1927) that fungi take no active part in the destruction of organic substances under waterlogged conditions, even where oxygen has free access to the substances. Among the varied microflora of the sea-exposed fabrics comparatively few types of fungi occurred and those found were never present in appreciable numbers.

Even more striking was the absence of actinomycetes which were found in only small numbers represented by some six different types as against 23 types of fungi and 125 types of bacteria and cocci.

In no case were algae detected on the exposed fabrics, though a species was isolated from the harbour mud.

Of the bacteria and cocci found, by far the largest number were types of what may be described as a "secondary microflora". Nearly all of them grew well on ordinary bacteriological media; they failed to attack cellulose, either lignified or unlignified, while some of them were able to decompose wool. Of types capable of decomposing cellulose in the form of filter paper, some grew most readily under mesophilic aerobic conditions, others at thermophilic aerobic temperatures. Others again grew best under anaerobic conditions either at 30–37° or at 60° C.

Of special interest was a group of spore-forming rods of the *Clostridium* type which was frequently met with on the various cellulosic fabrics; they grew particularly well in media containing hemicelluloses such as xylan. This particular carbohydrate was broken down by them with the evolution of gas and the production of undetermined alcoholic compounds. It was noticeable that these hemicellulose-decomposing types were much more numerous on the exposed fabrics than in the mud from the harbour.

It was mentioned above that the supervisor of the exposure tests at Colombo Harbour had remarked on the rapid rate at which the exposed fabrics decomposed. When similar samples of cotton materials were exposed in the sea at the station in England the rate of destruction was undoubtedly slower, with from 50 to 75 % of attacked fibres at the home station after 27 days' exposure against 98–100 % at Colombo. The temperature of the water at the latter station during the period of exposure had been 26·5–28 against 14–16° C. at the home station. It is possible, therefore, that the greater rate of attack at Colombo may have been due to the higher temperatures prevailing there, though it cannot be excluded that particularly active cellulose-destroying bacteria may have been present at Colombo. The reason for the rapid destruction of the woo

fabric exposed at Colombo cannot be interpreted on the available evidence since it was found impossible to establish that micro-organisms were the sole cause of decay. It has been mentioned already that Dorée had found that wool fabrics were more resistant to deterioration in salt water than cotton: at Colombo Harbour the reverse was the case.

Summarizing the observations made on fabrics which had been exposed under waterlogged conditions the claim appears justified that micro-organisms are the sole agency responsible for the decay of cellulosic materials exposed to such conditions, at any rate when the relevant organisms are present, either on the exposed fabrics or where they have ready access to the fabrics.

A further conclusion may be drawn from these tests, namely, that fungi, actinomycetes and algae play no conspicuous part in the destruction of cellulose fabrics under waterlogged conditions. Whether the mere saturation of fabrics with water under aerobic conditions, perhaps in combination with diffused light, causes a destruction of fabrics in the absence of direct contact with sources of infection, is a question of some practical interest to the laundering industry, since it is held there that clothing, when it cannot at once be dried, is better protected against damage ("spotting") by being completely immersed in clean water than by being allowed to remain damp.

In theory, Dorée's investigations (1920) would appear to answer the question, since prolonged exposure of fabrics in sea water containing antiseptics failed to cause deterioration. But when no antiseptics are present and when an opportunity therefore exists for a destruction of the fabrics to take place, it would seem possible for infection, carried either by the fabric itself or by soil and dust particles, to initiate decay. However, since fungi appear to have little action on totally submerged fabrics, it is possible that "spotting", which is often held to be caused by "mildew" fungi, may be largely prevented by total submersion of fabrics in water.

(b) *Exposure of fabrics to damp, but not waterlogged, conditions*

Both "mildewing" and extensive destruction are known to occur in all types of fabrics which become damp. Burns (1927) states that a moisture content in cotton exceeding 11 % is sufficient to initiate deterioration and mentions that where 20 % of water is added to raw cotton "heating" and destruction set in. Similar moisture contents would appear to initiate deterioration in flax (Ruschmann, 1923). Wool, on the other hand, does not seem to suffer damage through damp,

beyond "mildewing", until moisture contents of over 24 % have been reached.

Conditions of damp capable of introducing and maintaining 20–30 % of moisture in a fabric are likely to be met with in the soils of most climates and in the shaded humid atmosphere of certain tropical countries.

In exposure trials carried out in Dorset, England, the writers found that cotton fabrics, left in the soil for 5 weeks, had absorbed and retained sufficient water to establish in them a moisture content of over 30 % in spite of the fact that the soil itself never exceeded 15·5 % while the fabrics were in contact with it. Similar and sometimes even more striking figures were obtained in fabrics exposed at other stations. They leave little doubt that in soils of most countries, and presumably in the damp humid atmospheres of some tropical climates, fabrics must be able to absorb moisture to an extent which would be fully sufficient to induce "mildewing" and destruction.

The examination which the writers carried out on cotton, flax, hemp, wool, silk, rayon and jute fabrics, exposed, in the case of the first four, in the atmosphere at Ceylon and Trinidad, and in the soil at Cyprus, Kenya, South Africa and Trinidad; and for the whole range of fabrics at the home station in Dorset, England, confirmed that "mildewing" would set in under the chosen conditions.

The destruction of the fabrics was found to be much more marked in the soil-exposed samples than in those hung in the air at the places of exposure. Columns 6 and 7 of Table I illustrate this.

Table I. *Fabrics exposed at Peradeniya, Ceylon*

Description of sample	Duration of exposure		No. of micro-organisms found per g. of exposed fabric		% of fibres damaged by micro-organisms	
	Shade days	Soil days	Shade	Soil	Shade	Soil
American cotton fabric	365	30	$9·3 \times 10^6$	48×10^6	35	88
Egyptian cotton fabric	365	30	$4·3 \times 10^6$	$7·8 \times 10^6$	40	92
Indian cotton fabric	365	30	$21·7 \times 10^6$	$6·5 \times 10^6$	46	92
Dew-retted flax fabric	365	58	$12·4 \times 10^6$	6×10^6	Approx. 50	Most of fibres damaged
Hemp fabric	365	58	$10·4 \times 10^6$	$1·1 \times 10^6$	Most of fibres damaged	Most of fibres damaged
Wool fabric*	365	30	$3·7 \times 10^6$	$2·7 \times 10^6$	No signs of damage	A certain* amoun of damage t fibres
Soil sample from place of exposure	—	—	—	$4·5 \times 10^6$	—	—

* As mentioned in the first section of this paper, microbiological damage cannot be assessed in the case o wool with the same certainty as in the case of cellulosic fabrics.

Table I shows that the damage to the fibres of the cellulosic fabrics was due to micro-organisms. This claim is based on the result of an analysis of the samples by the swelling test of Fleming & Thaysen (1920) and the Congo red test of Bright (1926).

Little difference was found in the rates of destruction of the various soil-exposed samples between those buried in the soil of a temperate climate such as Dorset, England and those in the tropical climate of, e.g. Malaya. Too far-reaching conclusions should not, however, be drawn from this observation, for the soil exposures at Dorset were carried out during May to August at a time when the soil temperature there did not fall below 15° C. In numerous other trials which the writers have carried out it has been noticeable that the rate of "mildewing" and destruction of a fabric buried in the soil is greatly slowed down during the winter months.

The visible changes suffered by the cellulosic fabrics exposed in the soils and by those hung in the shade were not dissimilar. "Mildewing" was apparent in both cases though decidedly more so in the air-exposed samples than in those from the soil. On the other hand, deterioration with loss of tensile strength was far more serious in the soil-exposed samples than in those hung in the air (see Table I). It is not explained in the table, however, or in any other direct experiment which has been carried out, why soil exposure should be so much more damaging to fabrics than shade exposure, while "mildew" is visually more noticeable on the shade samples than on those from the soil. Though an explanation based on experimental evidence cannot be offered for this, indirect evidence points to differences of moisture conditions in the fabrics as the governing factor.

It has been mentioned that tests had shown that cotton fabrics buried in a soil in Dorset absorbed sufficient water to reach a water content of over 30 %, a figure which according to Burns (1927) is more than sufficient to cause "heating" in raw cotton, a process of destruction which leads to a rapid disintegration of the textile properties of the cotton hairs, and which is associated with the growth of cellulose-destroying bacteria (Thaysen & Bunker, 1927). It is questionable whether the bacteria could grow on fabrics with moisture contents below 20–25 %. Unpublished experiments which one of the writers (H. J. B.) has carried out indicate that they could not.

Fungi, on the other hand, have been found by the writers (in unpublished experiments) to cause "mildewing" on cellulose pulps containing no more than 10 % of moisture. It is not surprising, therefore,

that fabrics exposed in the air at relative humidities of such an order that they are capable of establishing a moisture content of 10 % or more in an exposed fabric should become "mildewed". However, since growth of fungi on a fabric with resultant "mildewing" causes a slow deterioration of the fibres composing the fabric, it is not surprising that even heavy "mildewing" may have failed to deteriorate a fabric to the extent of one exposed to attack by cellulose-destroying bacteria.

After 10 months' exposure in the shade at St Augustine, Trinidad, the test fabrics were weakened considerably more than those exposed at Kuala Lumpur in the Federated Malay States and at Peradeniya, Ceylon, though the actual numbers of micro-organisms found on the Trinidad samples, for instance, were higher than those found on the Ceylon samples.

Here again, differences in humidities at the places of exposure can be correlated with differences in loss of tensile strength, at any rate for the two stations, Trinidad and Malay States, for which data are available. This may be seen from Tables II and III.

Table II. *Relative humidity*

	Federated Malay States		Trinidad	
	Morning	Evening	Morning	Evening
Av. for month	%	%	%	%
Jan.	87	83	92	66
Feb.	87	87	93	55
Mar.	91	83	96	57
Apr.	84	87	95	54
May	80	88	96	64
June	84	87	95	63
July	84	91	95	72
Aug.	—	96	95	84
Sept.	84	91	96	79
Oct.	83	87	93	77
Nov.	83	—	95	78
Dec.	—	—	94	74

Table III. *Fabrics exposed in the shade*

Type of fabric	Federated Malay States % of damaged fibres	Trinidad % of damaged fibres
American cotton	62	19
Egyptian cotton	85	15
Indian cotton	60	33
Dew-retted flax	59	A small % of total

In connexion with another investigation, one of the writers (H. J. B.) ascertained the moisture content of chemical wood pulp kept for a month at varying relative humidities. The results are shown in Table IV.

Table IV

Approx. relative humidity of atmosphere %	Approx. equilibrium moisture content of pulp	
	At room temperature %	At 30° C. %
98	30	21–22
90	13·5	11
80	10	8·5–9·0
70	8–8·5	7·5–8·0

It is clear that at Trinidad the relative humidity of the atmosphere must frequently have been of an order which caused the exposed fabrics to dry to an extent which reduced the moisture of the fabrics to below the minimum concentration at which fungi can develop. This minimum was estimated by Fleming & Thaysen (1921) as 9 %, and by Armstead & Harland (1923) as 7·8 %.

The wool samples exposed under the conditions discussed here, i.e. under damp but not waterlogged conditions, were far less affected than those immersed in sea water. A certain harshness was noticeable in the shade-exposed samples, but no tendering of the fabric was noticeable. The wool fabrics exposed in the soil had been tendered and broken up in the manner observed when wool-destroying bacteria act on wool under laboratory conditions.

The silk and rayon fabrics exposed in the soil were all rapidly destroyed, with the exception of the acetate rayon, which remained unchanged during the year of exposure. This high resistance was very noticeable indeed.

The soil-exposed jute fabric suffered rapid disintegration in spite of being partially lignified. The damage caused to the jute could be correlated, as in all the other soil-exposed cellulosic fabrics, with the presence of a large number of micro-organisms, including cellulose-destroying forms.

The types of micro-organisms present consisted chiefly of forms which had no action on cellulose and, therefore, must be regarded as a secondary microflora. Cellulose-decomposing types were present in all the soil-exposed and in some of the shade-exposed fabrics. Their numbers could not be determined quantitatively.

Few actinomycetes were isolated from the shade-exposed samples and these forms obviously did not play an important part in the changes which took place in the fabrics during exposure. Fungi were very much in evidence on the shade-exposed samples, but not in the soil samples. No algae were found. Where "mildewing" or spotting had occurred fungi

could be isolated which, when grown on sterile damp fabrics, could be made to produce such "mildewing" and spotting.

(c) *Exposure of fabrics in climates with moderate humidities*

Detailed records are available of the moisture gain of the various fabrics exposed in the shade at Dorset. Reference to Table V shows the

Table V

Station	Total rainfall in in. for the year of exposure	Max. relative humidity in %	Min. relative humidity in %	Av. relative humidity for the year of exposure in %		
				Morning	Noon	Evening
Adelaide	22·54	81	30	53	42·8	64·1
Dorset	28·35	100 (recorded once)	21 (recorded twice)	81·7	—	73·5
Nairobi	26·68	90·7 (recorded once)	36·3 (recorded once)	86·7	55·5	77·8
Pretoria	34·92	84 (recorded once)	20 (recorded once)	77·3	59·0	54·0

moisture conditions in shade-exposed samples which prevailed at the four stations during the year of exposure. In Dorset the moisture found in the three types of exposed cotton fabric never exceeded 20 % and only reached this figure on one occasion, in an Indian cotton fabric. On the days of removal for analysis, all the other samples had moisture contents ranging from 4·7 to 11·2 %. The reason for this great variation is undoubtedly the length of time which elapsed between the last heavy fall of rain and the removal of the sample for analysis. By consulting the relevant data in the records it can be seen, for instance, that the sample of flax fabric which was removed after 9 months' exposure in the shade had been exposed to a fall of rain measuring 19·8 mm. 5 days previously, and that within those 5 days the moisture content of the fabric had been reduced by air-drying from complete saturation to 10·4 %. In the case of another fabric, small falls of rain of the order of a few tenths of a millimetre failed to increase the moisture content beyond 12 %. It is evident, therefore, that except on days of heavy rain the moisture content of the shade-exposed fabrics must have been fairly low, probably of the order of 10–12 %; such concentrations according to Burns (1927) are on the border line of those capable of initiating growth of micro-organisms on textiles.

This was confirmed by the analysis of the various fabrics. In spite of a full year's exposure they contained few micro-organisms, and the damage was restricted to "mildewing" or spotting. Certain types of fungi could be detected in the damaged places. The spotting was more

noticeable on the exposed cotton fabrics than on the heavier linen, hemp and jute fabrics. The viscose rayon fabrics were also extensively spotted, and even the acetate rayon showed occasional spots. In the latter case, however, no fungi could be seen in the spots, which were composed of a dark yellow gum containing large cells, possibly algae. Spotting of a less marked nature than on the cotton fabrics was also met with on the exposed silk and wool fabrics, but this damage can have been of little significance for no fungus mycelium was found in them, and the hairs and fibres of the fabrics were sound.

The bacteriological analyses of the samples revealed much lower numbers of micro-organisms than in fabrics exposed in damper climates. The reduction in numbers was particularly noticeable among the bacteria. It is evident, therefore, that prolonged exposure of fabrics in the shade at places of comparatively low average humidities, between 50 and 90 % R.H., and with normal annual rainfalls of about 30 in., offers little opportunity for micro-organisms to develop, even in warm climates. Where microbiological growth becomes macroscopically noticeable it is restricted to a few species of fungi which cause pigmentation, but no lowering of the tensile strength of the fabric. The spread of these fungi appears to be greater on the cotton and rayon fabrics than on the heavier fabrics of flax, hemp and jute. Wool and silk fabrics are little affected by them.

A certain harshness was observed on the exposed wool fabrics. This could not be associated with microbiological activity but resembled the initial stages of the type of deterioration suffered by this fabric on exposure to sunlight.

(d) Exposure of fabrics in the shade in climates with low relative humidities

This type of climate was represented by the station at Broken Hill South, N.S.W., Australia. The total rainfall during the year of exposure here was 9·56 in., with a yearly average humidity of the air of 57·6 % at 6 a.m., 40·5 % at noon, and 39·1 % at 6 p.m. Twice during the year the relative humidity rose to 100 %, but it usually deviated little from the yearly average. The minimum for the 6 a.m. reading was 17 %, for the noon reading 9 %, and for the 6 p.m. reading 11 %.

It was surprising to discover in the analyses of the fabrics that the total number of micro-organisms found was of the order of that of fabrics exposed at stations with moderate rainfalls and humidities between 50 and 90 %. Nevertheless, none of the exposed fabrics had

suffered any damage either through loss of tensile strength or through discoloration—"mildewing". The wool fabric had become slightly harsh but a microscopic examination showed that the hairs were normal. Among organisms found, the secondary flora of harmless bacteria and cocci represented the bulk. Actinomycetes were almost invariably absent and fungi were few in number. Cellulose-destroying bacteria were only isolated from one of the exposed fabrics. Algae were not isolated. It may be concluded, therefore, that arid climates offer no facilities for the "mildewing" and destruction of fabrics.

(e) *Exposure of fabrics to sunlight in climates with different relative humidities*

The series of exposures included a wide range of climatic conditions from the point of view of rainfall and intensity of sunlight. At Dorset, England, which probably represents the station with a minimum sunshine record, the daily average amounted to 4·6 hr.; at St Augustine, Trinidad, it was 5·6 hr., and at Adelaide 6·5 hr.

The total annual rainfall during the year of exposure is shown in Table VI.

Table VI

Station	Total amount of rain during year of exposure, in in.
Peradeniya (Ceylon)	89·57
St Augustine (Trinidad)	63·1
Pretoria (South Africa)	34·92
Dorset (England)	28·35
Adelaide (Australia)	22·54
Broken Hill South (Australia)	9·56

An examination of the results of the analyses of this series reveals several interesting facts. It appears, for instance, that "mildewing", the degree of microbiological damage suffered by the exposed cellulose fabrics, and the growth of the secondary microflora are all greatly influenced by the annual rainfall, rather than by the amount of sunshine at the place of exposure.

At Trinidad, for instance, where there was a daily average of 5·6 hr. of sunshine, there was very considerable microbiological activity on the fabrics, while at Dorset, England, with a daily average of 4·6 hr. of sunshine, the samples showed far less microbiological damage and lower numbers of micro-organisms than at Trinidad.

On the other hand, it must be admitted that sunlight exercised a considerable action on the microflora of the various fabrics. This is well seen

in a comparison of the shade- and the sun-exposed samples from Ceylon and Trinidad. The shade-exposed fabrics at both stations not only gave higher total numbers of micro-organisms, but a greater percentage of damaged fibres than the corresponding sun-exposed samples. "Mildewing" or spotting was more noticeable in the shade-exposed samples from all stations than in the corresponding sun-exposed fabrics.

In addition to this retardation of damage by sunlight, other effects were observed on the various fabrics. For instance, sun exposure rendered the wool, rayon and silk samples harsh to the feel. The tensile strength of the various cotton fabrics was reduced by the action of sunlight, and even the heavier flax and jute fabrics showed signs of loss of tensile strength after exposure to sunlight. In no case did this action of the sun, which presumably resulted in a degradation of the chemical constituents of the fibres, lead to an increased activity of micro-organisms.

Another general conclusion may be drawn from the analysis of the sun-exposed fabrics. Where microbiological damage, as determined by the swelling test of Fleming & Thaysen, was caused to the fabrics, it was invariably the result of the activity of fungi. In every case which was examined a discoloration—"mildewing"—of a fabric could be ascribed to fungus growth and it was at places of discoloration that a destruction of the fibres could be detected. This observation is corroborated by the almost total absence in the sun-exposed fabrics of cellulose-destroying bacteria and actinomycetes, and by the presence of an unusually small number of bacteria of what has been described as the secondary microflora.

5. THE EFFECT OF EXPOSURE ON THE VISCOSITY AND THE REACTION OF FIBRES AND FABRICS

It has already been mentioned that cellulosic and woollen fabrics, when exposed to the action of sunlight in certain tropical countries, suffered a loss of tensile strength due to a weakening of the fibres composing the fabrics. The damage could be shown by appropriate technique (Fleming & Thaysen, 1920) to be similar to the tendering suffered by fibres which had been exposed to the action of chemical agencies such as dilute inorganic acids.

When certain fabrics which the writers had exposed in Dorset, England, were examined, special attention was paid to the action of sunlight for the purpose of determining whether some effect, other than loss in tensile strength, could be detected. It was decided to determine,

on the one hand, the changes, if any, which might have occurred in the viscosity of the fabrics on exposure, and on the other, the changes in the reaction of the samples. It was argued that changes in viscosity
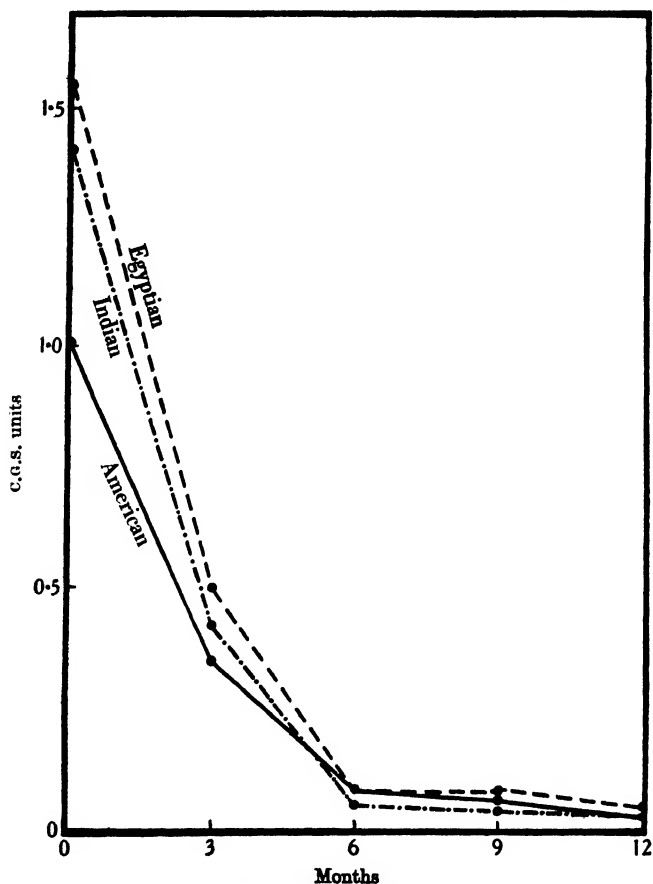
Text-fig. 1. Viscosity of cotton fabrics after exposure to the sun in Dorset, England. Viscosity measured by Clibben's method in c.g.s. units on a 1% solution. Viscosity of unsterilized samples of fabric: American cotton, 2·79 units; Egyptian cotton, 2·25 units; Indian cotton, 3·90 units.

without any alteration in the reaction of the fabrics might indicate an early stage in the breakdown of the cellulosic substance of the fibres, while changes in both viscosity and reaction would indicate a more advanced breakdown.

The work on the determination of the viscosity of the various cellulose fabrics exposed in Dorset, England, was carried out in collaboration with Dr Barr of the National Physical Laboratory, Teddington, and
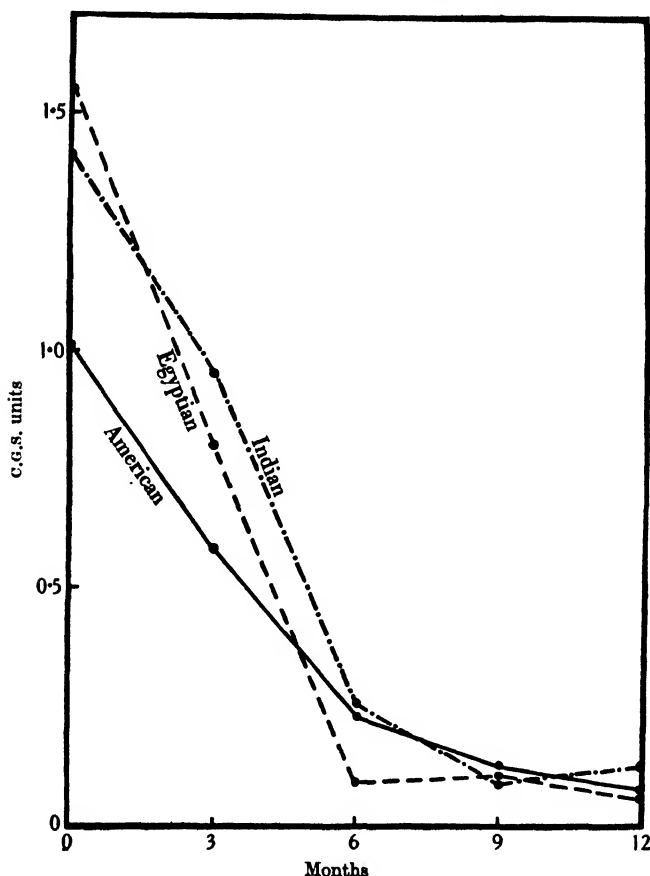
Text-fig. 2. Viscosity of cotton fabrics after exposure in the shade in Dorset, England. Viscosity measured by Clibben's method in c.g.s. units on a 1 % solution. Viscosity of unsterilized samples of fabric: American cotton, 2·79 units; Egyptian cotton, 2·25 units; Indian cotton, 3·90 units.

with Dr Gibson of the Linen Research Association, Belfast. Dr Barr kindly undertook to determine the viscosity of the cotton samples, and Dr Gibson of the flax and hemp samples.

The results of the viscosity determinations of three types of cotton

fabrics exposed in the sun, in the shade and in the soil are shown in Text-figs. 1–3.

In interpreting these figures it should be emphasized that the careful process of dry sterilization to which the fabric samples had been subjected before exposure (160° C. for 2 hr.) had, in the case of cottons, very materially reduced their viscosity. This reduction became even more marked during the subsequent sun and shade exposure, in the sun more rapidly than in the shade.
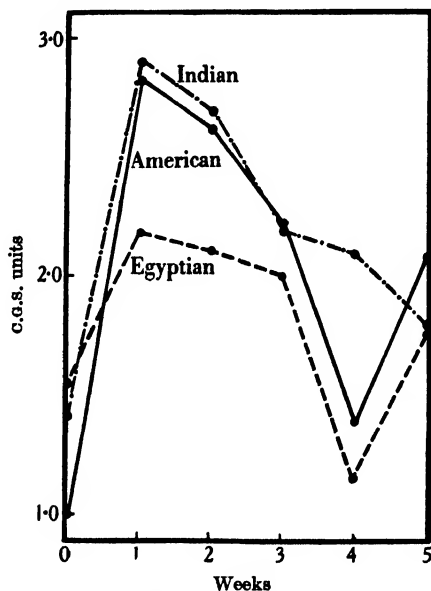
Text-fig. 3. Viscosity of cotton fabrics after exposure in soil in Dorset, England. Viscosity measured by Clibben's method in c.g.s. units on a 1% solution. Viscosity of unsterilized sample of fabric: American cotton, 2·79 units; Egyptian cotton, 2·25 units; Indian cotton, 3·90 units.

The soil-exposed samples behaved differently. Here there was an initial steep increase in viscosity which may perhaps be regarded as a recovery towards normal from the loss sustained during sterilization. The rise was followed by a slight decrease which is, perhaps, most representatively shown in the case of the Indian cotton. The final viscosity figures for the soil samples were in all cases higher than those of the original sterilized samples at a time when the samples had to be removed from the soil because of their extensive destruction by micro-organisms. This observation appears to imply that the destruction of cellulosic

fabrics by micro-organisms has comparatively little effect on the viscosity of the materials, an interpretation which is confirmed by the results of the viscosity changes in the exposed flax and hemp samples. Here sterilization had caused no lowering of the viscosity, and a subsequent drop was noticeable only in the sun- and shade-exposed samples, in the former more so than in the latter. The soil-exposed flax and hemp fabrics retained their original viscosity to the end of the trial at a time when the samples were extensively damaged by micro-organisms. These observations support the findings of Searle (1929), who states that flax fibres destroyed by micro-organisms have a viscosity of the order of that of normal fibres.

Two methods were adopted for ascertaining the changes in acidity of the cellulosic fabrics. The first gave a very rough indication of the pH value of the samples. It consisted in rubbing a drop of a suitable indicator into the fabric with the help of a glass rod, and observing the change in colour which occurred. The colour change was matched against a suitable standard set. The details of this method were communicated to the writers by members of the staff of the Shirley Institute, Didsbury, Manchester.

The second and more quantitative method consisted in extracting at room temperature 1 g. of a representative sample of the fabric undergoing test with 10 ml. of distilled water of pH 7·2. The extraction was continued for 10 min. and the liquid then drained off. The determination of the hydrogen-ion concentration was carried out on the extracts and was measured by suitable indicators. Any change in the pH values of the extracts was assumed to be due entirely to changes in the reaction of the fabric from which the extract had been prepared.

It was established that the preliminary sterilization which the fabrics had been given prior to their climatic exposure had caused no measurable change in their reaction, though, as mentioned above, sterilization lowered the viscosity of the cotton fabrics used.

Without giving details of the results, it may be claimed that all the sun- and shade-exposed samples, including even silk and wool samples, showed an increase in acidity during the first 3 months of exposure. This increase was sufficient to lower the pH values of the extracts from the original pH 6·0 in the case of cotton and 5·0 in the case of jute, to 5·0 and 3·8 respectively in the sun-exposed samples, and to 5·0 and 4·5 in the shade-exposed samples. Subsequent exposure gave rise to little further change, the figures for 3 months being maintained throughout the year of trial.

Soil exposure caused practically no change in the pH values of the extracts, indicating that the extensive microbiological damage suffered by the cellulose of the fabrics failed to yield acidic products in measurable quantities or sufficient to prevail over the neutralizing action of the soil itself.

It is not possible to give an explanation for the cause of the increase in acidity of the sun- and the shade-exposed samples, but the fact that this increase had almost reached its maximum within 3 months of exposure at a time when very little damage had been caused by light must imply that light exposure at relative humidities such as those prevailing at the station where the trial was carried out fails to cause a breakdown of cellulose to such an extent that acidic decomposition products result.

6. ON CERTAIN FACTORS WHICH INFLUENCE THE RESISTANCE TO DECAY OF FABRICS EXPOSED TO MICROBIOLOGICAL DETERIORATION

When the moisture content of a test fabric, as well as the moisture of its surroundings, was kept above 9%, and when the prevailing temperature and other relevant factors such as the place of exposure were maintained constant, it was possible to anticipate that two samples of a given fabric or even samples of closely related fabrics would become affected by micro-organisms at a similar rate and to a similar extent. Cases were not lacking, however, where this did not appear to hold good. The exploration of such cases has led to conclusions which are of a certain practical significance, and merit record.

In the course of an examination of cellulosic fabrics which had been exposed in the soil at Dorset, England, it was found that a viscose rayon fabric of a light voile texture had rotted completely after 3 weeks' exposure, while a heavier and more closely woven material of the same fibre lasted for 4 weeks in the same soil and at the same season of exposure. This and similar observations on other fabrics was interpreted as indicating that the rate of deterioration in soil is inversely proportional to the weight of a fabric, the heavier the fabric the longer would the greater part of its tensile strength be maintained.

Other experiments indicated that additional factors might influence the rate of destruction of fabrics even when moisture conditions, temperature and microflora remained constant and favourable. It was found, for instance, that a sample of loosely woven cotton fabric exposed · in the soil at Dorset had lost practically the whole of its tensile strength

after 40 days, while a sample of much more closely woven cotton duck of approximately double the weight lasted for 126 days, that is, more than three times as long as the loosely woven material. The apparent explanation for this, that the outside layers of cotton hairs in the closely woven material prevented moisture from penetrating to the interior of the fabric, could not on further investigation be accepted as satisfactory. For it was found that where a piece of cotton fabric 20×15 cm. was protected on both sides by a shield of glass to prevent moisture penetrating into the fabric, the water content of the material exposed in the ground for 28 days rose to 62 %, while an unshielded control sample of the same material showed only 46 % of moisture when kept under identical conditions. And yet, in spite of the greater water content, the glass-shielded material had retained most of its tensile strength, while the sample of the same cotton fabric exposed in the ground to direct contact with the soil had disintegrated.

This observation suggested further experiments on the effect of shielding of exposed fabrics. Such experiments confirmed that shielding of a fabric by rubber, by a metal such as aluminium, or by glass, made the protected material retain its tensile strength longer on exposure in soil or in the sea than unshielded material of an identical description.

The degree of protection afforded by shielding was determined in a set of soil exposures with a light cotton cloth. This was left partly unshielded in the control sample, or shielded by aluminium, glass, rubber, or vaseline. The various samples were buried vertically in an active garden soil under identical conditions. The temperature of the soil throughout the exposures varied between 70 and 10° C.

The result of this trial is shown in Table VII.

Table VII

Nature of shielding applied to the fabric	No. of days' exposure in the test soil required for the fabric to deteriorate sufficiently to be torn readily by hand
No shielding	28
Shielded with glass	119
,, aluminium	105
,, rubber	105
,, vaseline	32

The moisture content of the shielded fabrics (glass and aluminium) after 28 days' exposure was 15·8 and 16·8 % respectively. After 90 days it was 49 and 44 % respectively. Thus, the shielding of a fabric by inert substances can be shown to retard the normal rate of deterioration of

a fabric by micro-organisms. A similar effect, it may well be assumed, could have been responsible for the great resistance to decay which was shown by the closely woven cotton duck referred to above. The outer layer of hairs or even of threads in this and similar closely woven heavy fabrics may well have acted as a shield protecting the bulk of the interior hairs or threads against decay, and thus reducing the rate of deterioration of the fabric taken as a whole.

Some consideration has been given to the problem of the mechanism by which inert substances are capable of protecting exposed fabrics. It is evident from what has been said that the protection given by shielding cannot be due to an elimination of moisture. Most other conditions which govern the destruction of fabrics by micro-organisms can also be eliminated as a cause, with the exception of the direct contact between an active source of infection and the shielded fabric.

Most workers who have studied the subject of cellulose decomposition have been able to demonstrate that intimate contact between cellulose-digesting micro-organisms and the material which they are expected to attack is essential. It has been suggested (see Thaysen & Bunker, 1927) that the need for this intimate contact is due to the failure of the enzymes produced by cellulose-destroying micro-organisms to function at a distance away from the living cells producing them. Moreover, for a rapid contamination of fresh cellulosic material and for its quick destruction it is essential that it should be in close contact with a large number of active cellulose-destroying micro-organisms. Such large numbers of active cells are, in practice, limited to decaying cellulose debris, the surface of which is covered with masses of cellulose-destroying organisms which adhere firmly to their substrate and thus overcome the difficulty of the limited range of action of their enzymes. When such direct contact is prevented by the introduction of an inert substance between the contaminated cellulose debris of the soil and the fabric to be attacked, infection can occur only through free cellulose-decomposing organisms present in soil waters which may seep into the fabric with the moisture. Since comparatively few such cells are found in soil water the infection of a shielded fabric will progress slowly, particularly as a large amount of growth has to establish itself on fibres before a noticeable loss of tensile strength can be recorded.

That intimate contact between soil and an exposed fabric is established before rotting sets in can easily be observed. An unprotected fabric placed in or on the soil will be found within the first 2 weeks to have a great many particles of soil adhering tenaciously to it. An

effectively protected fabric, on the other hand, can be removed from its place of exposure without adhering soil particles even after many weeks' exposure. Some soil may have become mechanically entangled, but this can easily be removed by gentle shaking.

From time to time preparations have been offered commercially as protections against the rotting of fabrics, the sole function of which has clearly been one of shielding. As typical examples may be mentioned rubber latex and other types of pure rubber compositions, waxes, linseed oil and aluminium soaps. In no case have the writers found such preparations to afford more than temporary protection to a fabric. Their useful application, therefore, must necessarily be limited.

A different type of shielding is met with in jute fibres which are surrounded by parenchymatous and epidermal tissue, sufficiently coherent and continuous to retard the action of such drastic swelling agents as strong alkalies. Hemp may sometimes show such tissues around the fibre bundles, while flax, where badly scutched, can occasionally be seen to have fragments of parenchymatous tissue adhering to the fibres. Cotton hairs are not shielded in any way.

The various exposure trials which the writers carried out with fabrics at Dorset, England, offered an opportunity to determine the protection afforded by the parenchymatous tissue of jute. The results obtained are tabulated in Table VIII, which includes also data on the rate of deterioration of one hemp, two flax and a cotton fabric exposed with the jute fabric under identical conditions in an active garden soil.

Table VIII

Type of fabric	Wt. of fabric in g. per 100 sq. cm.	Actual no. of days taken for the fabric to deteriorate to an extent which allowed it to be torn readily by hand	Calculated no. of days taken for the standard degree of deterioration to be reached when assuming that deterioration would be proportional to weight of fabric and expressing this weight as the strength of the dew-retted flax sample
Dew-retted flax	4·42	20	20
Water-retted flax	4·42	20	20
Hemp	4·42	21	20
Jute	7·47	35	21
Cotton	1·78	14	35

It will be seen that flax, hemp and jute rotted at an almost identical rate while cotton deteriorated somewhat more slowly. The latter conclusion is probably not very significant since the result was obtained on a much lighter fabric than those of the other fibres. Nevertheless, the

results obtained demonstrate sufficiently clearly that the parenchymatous sheath of jute fibres fails to function as a protection against micro-biological attack. Incidentally the results fail to support the view some-times expressed that flax and hemp fabrics are more resistant to rotting than cotton and that hemp "is a very durable fibre which is not rotted by water" (Matthews, 1924). Nor do the results indicate that the lignin of jute (Cross & Bevan, 1908) is able to delay the rotting of these fibres beyond the time taken for fibres of pure cellulose.

7. On the methods available for the protection of fabrics exposed to climatic conditions

A perusal of the relevant literature gives the reader the impression that the two quite distinct problems of the "mildewing" of fabrics on climatic exposure and of the corresponding damage by fungus attack during manufacture, storage and transit, can be treated as one. Both admittedly are essentially of a microbiological nature, but whilst the former deals with a host of micro-organisms comprising bacteria, fungi and actinomycetes, the latter is restricted to the kinds of lower fungi (Bright, 1925) which may appear on fabrics in the textile mills during manufacture, storage and subsequent transit; Galloway (1930) has given a list of fungi isolated by him from cotton goods. Experience has shown that in many cases the lower fungi can be checked by incorporating a suitable fungicide into a fabric, an expedient which would often fail to influence the progress of damage during climatic exposure, since active fungicides are frequently poor bactericides. There is the further difference between the two problems that climatic conditions will expose a fabric to agencies which, while unimportant during manufacture and transit, may seriously influence resistance to attack by micro-organisms. Exposure to water for instance will leach out of the fabric and eliminate water soluble substances with fungicidal or bactericidal properties. Exposure to heat and wind will aid evaporation of volatile inhibitory substances, and the action of actinic rays may induce changes in these substances which render them less active. Apart from the increased complexity due to exposure to a much greater variety of micro-organisms, climatic exposures, therefore, are much more exacting than exposure to con-tamination in the mill, and should be dealt with as a separate problem.

Very few instances are known to the writers of the prevention of microbiological attack on fabrics having been attempted by methods other than the incorporation into the fabric of toxic substances. The difficulties already outlined would require such substances to be insoluble,

or at any rate very little soluble, non-volatile and unaffected by the action of actinic rays. A further requirement would be the possession of both bactericidal and fungicidal properties.

The need for the use of only slightly soluble protectives implies that the substances used will function only in the closest proximity to the place of deposition. It is necessary, therefore, that it should be evenly distributed, not only on the surface of the fabric but on every individual fibre and part of fibre composing the treated fabric. In none of the commercial preparations which have been examined have the writers found this requirement fulfilled.

The uneven distribution of the protective agents on a treated fabric is well illustrated in Pl. XLII, figs. 1–4. The specimens were taken from samples of cotton duck which had been treated (1) by the cuprammonium process, (2) by incorporation of copper oleate, (3) by the iron-chromium treatment, and (4) with rubber latex.

In the case of the material treated by the iron-chromium process it was necessary to photograph the fibres at a comparatively high magnification in order to bring out the uneven distribution of the active substance of deposited iron-chromium on the fibres themselves. Text-fig. 3 shows that the bulk of the fibres have perfectly clean and obviously unprotected surfaces.

The protection afforded by rubber is due to "shielding", a mode of protection which was referred to in some detail in § 6. How ineffective the shielding has been in this particular case can be seen in Text-fig. 4, which shows large parts of each thread of the fabric completely free from the black dyed rubber.

In the cuprammonium, and notably in the copper oleate, treated samples of cotton duck penetration has been better, though unprotected parts of the yarn can still be detected. This, no doubt, is due to one thread being superimposed on another, thus preventing the protective substance from reaching the lower thread.

Before an attempt can be made to assess the degree of protection which is afforded by the incorporation into a fabric of toxic substances it is necessary to devise a technique for doing so. Such technique should give the highest degree of accuracy in evaluation, be reproducible, and be completed in the shortest possible time. A further requirement, which in the writers' view is important, is that it should approach as closely as possible the most exacting conditions to which a fabric is likely to be submitted, including leaching, evaporation, aeration, and the interaction of the complex microflora met with under such conditions.

In practice a very great number of tests for determining the resistance of protective substances have been carried out under natural climatic conditions. In this way fishing net and tentage preservations have usually been evaluated. With all its advantages, however, such a method suffers from the serious drawback that the direct results obtained cannot readily be compared since they are often materially influenced by the temperature, and on occasions also by the humidity prevailing at the site of exposure. Thus fabrics exposed in a tropical soil might be destroyed more rapidly than the same fabrics exposed in a temperate climate. Even results obtained at one station in a temperate climate might be found to vary with variations in the seasons of the year. The writers' experience is that this difficulty can largely be overcome by expressing the results, not in terms of actual number of days, weeks or months during which an exposed fabric resisted destruction, but in terms of the ratio between the times taken by a treated and an untreated sample of the same fabric, exposed under identical climatic conditions, to reach the same degree of deterioration. This degree may be chosen arbitrarily, but should be of an order which excludes any doubts as to its nature, whether microbiological or not.

Some workers (Thom *et al.* 1934) have used less comprehensive tests, but these can be justified only when the results obtained can be correlated with the results of exposure to actual climatic conditions.

Searle (1929), like Thom and his collaborators, stresses the importance of the time factor in exposure tests, and for this reason rejects the method of natural exposure in favour of one which can be completed in a shorter time. His technique retains the principle of exposure to the action of soil. Standard-sized strips of the fabric to be tested are brought into close contact with a thin layer of a fine suspension of soil particles distributed on the surface of a Berkefeld filter. The test fabric samples, after being wrapped round the infected moist filter, are incubated under humid conditions at constant temperatures and their tensile strengths determined on a Goodbrand machine at intervals of from 1 to 3 weeks or more. The method, according to Searle, gives consistent results in a shorter time than most natural exposure tests. In the writers' opinion it does not fulfil all the requirements of a reliable test and should not be used, without further exploration, to predict the behaviour of a rot-proofed fabric under climatic conditions. Still more arbitrary are the results obtainable by the methods proposed by Levine & Veitch (1920) and by Thom *et al.* (1934). Both are based on the use of fungi as the only agency of destruction during exposure, in Levine's test of a mixture of

fungi, in Thom's test of one single type. However standardized it is possible to make such methods as regards humidity, temperature and time, they fail to take into account the many other factors which influence deterioration under climatic conditions, and must remain arbitrary until the relationship between them and actual climatic exposure has been established.

The impression which the writers have gained from their work in this field is that too much emphasis has been laid on the importance of the time factor in the elaboration of tests for the evaluation of protective substances. There is much evidence in support of the statement in § 4 (b) that but slight differences can be observed in the rate of destruction of soil-exposed samples of one cellulosic fabric whether they be buried in the soil of a temperate or a tropical climate, provided that the soil temperature does not fall below 15° C. and that the soil possesses an active cellulose-destroying microflora. Under such conditions extensive rotting of untreated cellulosic fabrics will take place in 4–5 weeks, a period which is not very much longer than the time required in Searle's tests. Where exposure is carried out in a temperate climate during the winter months, using heavy fabrics for the purpose, the rotting process will naturally be slower.

Further work on the question of standardizing the conditions for a satisfactory technique for the evaluation of rot-proof substances will need to take account of these observations. The difficulties caused by one of them, the weight of the fabric, can easily be overcome by standardizing the type of material used in the tests.

To the writers' suggestion that tests for the evaluation of protective substances should be carried out by exposure of the proofed fabric in an active soil, the objection has been raised that such procedure is unnecessarily drastic; in fact more drastic than conditions likely to be met with in practice. That this is not so has been indicated in the preceding pages, but it may perhaps be pointed out that though a fabric may not throughout its active life be exposed continuously to such drastic conditions, occasions may well arise when it will be; for instance, sand bags and ground-sheets in direct contact with the soil, and fishing nets when left uncleaned on board a trawler after fishing operations have ceased. If a protective substance cannot withstand such conditions it cannot truly be claimed to be able to prevent microbiological destruction.

A considerable number of substances which from time to time have been recommended as protective have been evaluated by the writers by exposure to climatic conditions. Some of the results which have been

obtained in this way have been compiled in Table IX. Where not other-wise stated the test fabric used was a non-sized cotton material weighing 29·7 g./sq. ft. Control and treated material were suspended on aluminium frames measuring 12 × 12 in. and placed vertically either in the top foot of a good garden loam, or for sea exposures submerged below low tide in the sea near the shore.

The value of the several protective substances shown in Table IX is indicated in column 6. Here is recorded the ratio of the times taken for the control and the treated test fabric to deteriorate to the extent that it was possible to tear the fabric between two fingers of each hand im-mediately on its removal from the place of exposure. This may not be the most desirable method of determining the progress of the rotting of a fabric on exposure, and it is appreciated that in a finally standardized exposure test it may be desirable to replace this by a record of breaking load test on a Goodbrand machine. Nevertheless, the method adopted gave extraordinarily consistent results as may be seen from the data compiled in Table IX.

Table IX

Method of treatment	Soil exposure in days		Sea exposure in weeks		Ratio of destruction between control and treated fabric
	Untreated fabric	Treated fabric	Untreated fabric	Treated fabric	
Latex	—	—	17	35	1 : 2·1
Light yellow aromatic oil	—	—	10	14	1 : 1·4
Aluminium soap	49	49	—	—	1 : 1
Cutch treatment	—	—	10	13	1 : 1·3
Proprietary compound of cutch and chromium	66	158	—	—	1 : 2·4
Sodium silicofluoride	26	26	—	—	1 : 1
Xanthoxin	66	60	—	—	1 : 0·9
Proflavine	25	60	—	—	1 : 2·4
Acriflavine	25	60	—	—	1 : 2·4
2% diphenylamine fluorosilicate	26	40	—	—	1 : 1·5
Iron-chromium treatment of linen fabric	66	127	—	—	1 : 1·9
Iron-chromium treatment of cotton duck	127	235	—	—	1 : 1·9
Copper naphthenate	66	143	—	—	1 : 2·2
Copper oleate	—	—	25	71	1 : 2·8
Acetylation	45	>2645	—	—	1 : >58·8

Of the various materials chosen as protective substances the first three functioned probably through "shielding", the remainder in most cases through toxic action. The various treatments were tested either through sea exposure or soil exposure.

In only one case, that of acetylation, did the adopted treatment

increase the life of the test fabric by more than three times, a rather interesting observation in view of the extensive claims made for some of them. Acetylation was carried out by the method elaborated by Thaysen (1932, 1933, 1936). The results obtained by this treatment confirm those recorded by Dorée (1920) for cellulose triacetate fabrics, and indicate that cellulose acetates even of the order of the monoester or less, which have not suffered visible textile changes and retain their normal strength practically unimpaired, possess unrivalled resistance to destruction by micro-organisms.

That this resistance has been acquired by all the individual fibres or hairs of the treated cellulosic material and by every particle of each fibre is indicated by an experiment carried out on a closely woven cotton fabric which had been mechanically coated with a thin film of cellulose acetate. This fabric when buried under standard conditions in a soil was found to have rotted after 12 weeks' exposure. Examination showed that the active soil organisms had penetrated into the fabric from its ends, which had purposely been left unprotected. From there they had spread underneath the coating of the cellulose acetate layer which, though appearing macroscopically to cover the fabric with a continuous film, had been unable to envelop every thread and, even less so, every hair in the threads, both on its surface and throughout its lumen. It was ascertained that the coating of cellulose acetate on the fabric had not suffered through the exposure.

8. Summary

Various textile fabrics were exposed to a wide range of climatic conditions.

Those exposed to waterlogged conditions were destroyed rapidly. In the case of cellulose fabrics this destruction was due solely to bacteria, and not to fungi or actinomycetes. In the case of wool, the evidence of microbiological action was not conclusive.

Cellulose, wool, silk and cellulose rayon fabrics disintegrated on exposure in microbiologically active soils. The destruction was due to microbiological action, the rate being governed by the moisture content of the soil and, to a lesser extent, by the temperature. Cellulose acetate rayon was completely resistant.

Cellulose and wool fabrics exposed in the shade to very humid tropical conditions were damaged much less rapidly than in soil, although there was extensive "mildewing".

With fabrics exposed for a year in the shade at stations with sub-tropical or temperate climates, and with a rainfall of about 30 in., the microbiological damage suffered was insufficient to affect the strength of the fabric, but there was much "mildewing". No microbiological damage or "mildewing" was observed in shade-exposed fabrics in arid climates where the annual rainfall did not exceed 9·5 in.

Where fabrics were exposed to the action of sun as well as rain and wind, microbiological damage occurred when the moisture conditions were suitable for the growth of micro-organisms. This damage was invariably less than that of fabrics exposed in the shade at the same station.

Exposure of cellulose fabrics both in sun and shade caused a lowering in viscosity, whereas soil exposure, which causes more extensive micro-biological destruction, did not affect the viscosity. All sun- and shade-exposed samples showed a lowering in pH value during the first 3 months, but subsequent exposure caused little further change. Soil exposure caused practically no change.

Inert substances such as glass, metals and rubber, when used as a covering for cellulosic fabrics, retard microbiological decay in soil and sea exposures. Some commercial protective substances owe their value to their shielding action. The parenchymatous and epidermal tissues surrounding the fibre bundles of jute exert no shielding effect. In a micro-biologically active soil, flax, hemp and jute fabrics decay at approximately the same rate as cotton, which contains no protective tissue.

A provisional technique is described by which the relative merits of "mildew" protective treatments may be evaluated. The only treatment which has been found to give permanent protection in the case of cellulosic fabrics is partial acetylation.

The work described above was carried out as part of the programme of the Chemistry Research Board, and is published by permission of the Department of Scientific and Industrial Research.

REFERENCES

ARMSTEAD, D. & HARLAND, S. E. (1923). The occurrence of mildew in black-bordered dhooties. *J. Text. Inst., Manchr*, **14**, 475 T.

BRIGHT, T. B. (1925). Methods of examination of mildewed cotton material. *J. R. micr. Soc.* 141.

—— (1926). The microscopical examination of damaged cotton hairs by the Congo Red Test and the Swelling Test of Fleming and Thaysen. *J. Text. Inst., Manchr*, **17**, 396 T.

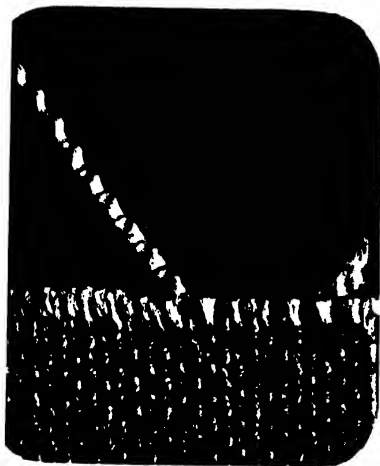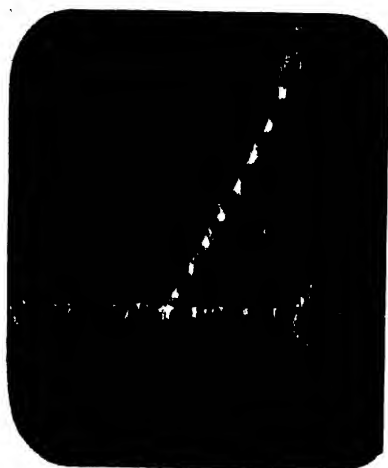BURGESS, R. (1931). Private information.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

THAYSEN, BUNKER, BUTLIN AND WILLIAMS.—THE EFFECT OF CLIMATIC EXPOSURE ON TEXTILE
FIBRES AND FABRICS (pp. 750–781)

BURNS, A. C. (1927). Deterioration of cotton during damp storage. *Bull. Minis. Agric. Egypt*, no. 71.

CROSS, C. F. & BEVAN, E. J. (1908). "Heart damage" in baled jute. *J. Soc. chem. Ind., Lond.*, **27**, 1129.

DORÉE, C. (1920). The action of sea water on cotton and other textile fibres. *Biochem. J.* **14**, 709.

FLEMING, N. & THAYSEN, A. C. (1920). On the deterioration of cotton on wet storage. *Biochem. J.* **14**, 25.

—— —— (1921). On the deterioration of cotton on wet storage. *Biochem. J.* **15**, 407.

GALLOWAY, L. D. (1930). The fungi causing mildew in cotton goods. *Shirley Inst. Mem.* **9**, 27.

LEVINE, B. S. & VEITCH, F. P. (1920). Testing the mildew resistance of textiles. *Bur. Chem., Leather and Paper Laboratory*, Washington.

MATTHEWS, J. M. (1924). *Textile Fibers*, 4th ed. New York: J. Wiley and Sons.

RUSCHMANN, G. (1923). Entwertung des Schwungflaxes durch Mikroorganismen. *Faserforschung*, **3**, 131.

SEARLE, G. O. (1924). A study of flax and kindred fibres. *J. Text. Inst., Manchr*, **15**, 370 T.

—— (1929). The rotting of textiles by micro-organisms. *Res. Inst. Mem. Linen Ind.* no. 56.

THAYSEN, A. C. (1932). Treatment of cellulose fibres and fabrics. Brit. Pat. No. 399,952.

—— (1933). Treatment of cellulose fibres and fabrics. Brit. Pat. No. 411,930.

—— (1936). Acetylation process for cellulose fibres and fabrics. Brit. Pat. No. 486,901.

THAYSEN, A. C. & BUNKER, H. J. (1927). *Microbiology of cellulose, hemicelluloses, pectin and gums*. London: Oxford Univ. Press.

—— —— (1930). Deterioration of fabrics by micro-organisms. Appendix V, *2nd Rep. Fabrics Res. Comm., Lond.* London: H.M. Stat. Off.

THOM, C., HUMFIELD, H. & HOLMAN, H. P. (1934). Laboratory tests for mildew resistance of outdoor cotton fabrics. *Amer. Dyest. Rep.* **23**, 581.

EXPLANATION OF PLATE XLII

Uneven distribution of protective agents on treated fabric (cotton duck).

Fig. 1. Treated by the cuprammonium process.

Fig. 2. Treated by the incorporation of copper oleate.

Fig. 3. Treated by the iron-chromium process.

Fig. 4. Treated with rubber latex.

ON THE LIFE-HISTORY AND ECOLOGY OF
LUMBRICILLUS LINEATUS MULL.
(OLIGOCHAETA)

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(With Plate XLIII and 3 Text-figures)

CONTENTS

INTRODUCTION

LUMBRICILLUS LINEATUS MULL. is abundant and widespread in its distribution, with a varied range of habitats. It is found on the seashore, in streams, polluted rivers and has been recorded from wells. It is also found in the bacteria beds of sewage works where its presence is particularly noteworthy, since recent investigations have shown that it influences the efficient functioning of the beds (Reynoldson, 1939). However, knowledge regarding the life-cycle of this organism is incomplete and its growing importance in applied biology and the ecological aspect of its extremely successful colonization of such a recent and unique environment as the bacteria bed make further information desirable.

The life-cycle has been studied throughout and the reactions of each phase examined experimentally under varying conditions of temperature to ascertain optimum conditions and limiting factors, and the results have been compared with observations in the field. Some study has also been made of the relationship of the worms to other animals in the beds. By this means the life-cycle of the worm has been recorded and evidence obtained regarding the conditions in the bacteria bed which have led to excessive multiplication of this worm.

HABITATS

General account

The Enchytraeidae are found in temperate and cold regions. Some groups live in the soil ("xerophilous", Stephenson, 1930a), others are typically hydrophilous types, whilst *L. lineatus* belongs to an important group most conveniently termed amphibious. It has the widest range of habitats recorded for any Enchytraeid and is the worm most frequently found inhabiting bacteria beds. It has been reported from these in different parts of England (Friend, 1916), from Belfast (Southern, 1909) and Chicago (Welch, 1914). Specimens in Chicago were named *L. rutilus* by Welch, but his description fits *L. lineatus*. This worm together with species of *Marionina* and *Enchytraeus* occurs in large numbers on the seashore near high-water mark, under stones and in rotting seaweed. They are able to withstand immersion in sea-water and must on occasion have to survive fresh-water. Schmidt (see Stephenson, 1930b) found *L. lineatus* in water of varying salinity from 4·5 g./l. upwards and the numbers were not reduced in water containing 39·62 g./l. This remarkable euryhalinity has probably contributed to the adaptability which enables this worm to exist in habitats which few organisms can tolerate. In addition, the severity of the competition for existence is reduced, enabling the worms to be abundant (Stephenson, 1930c). Friend (1916) has stated that the Enchytraeids with red blood are most frequently found in polluted localities where the oxygen is likely to be scarce and suggests that the greater respiratory efficiency of haemoglobin might be responsible.

The bacteria beds

A description of the bacteria beds, of the worm and its behaviour has been given (Reynoldson, 1939), so that only the most important points will be recalled. As a habitat for insects the beds have been described by Lloyd (1935) and for worms the following are the most important features: (1) the much reduced fluctuations in temperature of the beds compared with those of the atmosphere and most natural habitats; (2) the constant saturation of the beds with moisture; (3) the great depth of the habitable zone. In view of the abundance of the worms it is evident that these conditions, especially the temperature, must exercise a significant influence.

Methods

To study the life-history of the worm, observations were made twice weekly at the bacteria beds of the Leeds Sewage Works, Knostrop, from November 1936 to April 1938, and supplemented by laboratory investigations. For experimental purposes, the worms were kept in tubes, 3 × 1 in., fitted with corks provided with notches plugged by cotton wool for aeration. The bottom third of the tube was covered with black paper to provide protection against the harmful action of light and to discourage the worms from wandering up the tube where they otherwise often dried up. A pad of cotton wool, kept moist, was placed in the bottom of the tube. Small pieces of the alga *Phormidium* (*Cyanophyceae*), previously scalded, were used as food. Under these conditions, two worms could be maintained for weeks in each tube. By selecting worms with large ova ready for deposition, cocoons were obtained in 2–3 days. These were transferred to smaller tubes 1½ × ½ in. and kept on moist cotton wool. When hatched the young worms could easily be reared on a diet of the scalded alga.

The intensity of breeding was determined by ascertaining the number of sexually mature worms in collections from the beds, the approximate proportions of young worms present and also the approximate abundance of cocoons on the pebbles. Worms with well-developed clitella were taken as being sexually mature, although Stephenson (1930*d*) has stated that one part of the reproductive apparatus may develop whilst the rest lags. This however was not observed here.

Breeding

The breeding activity is apparently most intense during the colder months since at this time sexually mature worms predominated in the collections; young worms were common and cocoons abundant on the pebbles in the upper 6 in. This time agrees with that for the Enchytraeidae in general. There is no clearly defined season for breeding in *L. lineatus* and Oligochaetes in general as in many other animals, but merely a tendency for it to be more intense at one period of the year, and sexually mature worms are to be found at all seasons at Leeds. The reason for the existence of a seemingly more favourable period is at present undetermined. For some species of *Nais* it has been stated that oxygen production by algae was the effective factor, but this is not constant even for the Naididae (Stolte, see Stephenson, 1930*e*) and determining factors remain obscure.

Copulation was observed and found to agree with Ditlevsen's account (see Stephenson, 1930*f*). The two copulating worms lie head to tail, with the ventral surfaces in apposition and the penes which are the muscular terminal portions of the male ducts are inserted into the spermathecal apertures of the partner. The spermatozoa are introduced and stored in the spermathecae. When the cocoon secreted by the clitellum and containing the ova is carried forward over the spermathecal aperture, sperms are passed into it and the eggs fertilized. An unusual feature of *Lumbricillus* and other genera of the *Enchytraeidae* is the opening of the spermathecae into the oesophagus. It is supposed that any excess of sperm may be passed into the gut for digestion and absorption.

The cocoons

The cocoons are firmly attached to the substratum and this enables them to resist the constant downward trickle of sewage through the beds. They are deposited in the sheets of alga growing on the surface stones and upon the stones below, being most abundant on those with rough faces (Pl. XLIII, fig. 2). In the laboratory, the cocoons are found adhering to the strands of cotton wool, to the sides of the tube and to the pieces of alga.

The cocoons (Text-fig. 1) are ovoid and average $1\cdot1 \times 0\cdot75$ mm. but considerable variation was found, the length $1\cdot3$–$0\cdot9$ mm. and the breadth $0\cdot9$–$0\cdot56$ mm. By way of contrast the cocoons of *L. maximus* are much larger, being $1\cdot75 \times 1\cdot4$ mm. Thickenings of the wall occur at each end where closure has been effected by mucilage plugs during deposition. The wall of the cocoon is smooth and transparent and also composed of mucoid material. The ova are distinctly visible inside the cocoons as white, oval or rounded bodies and vary in number from 1 to 20 although 4–9 is the most usual. Ditlevsen records higher figures for this species (30–35). Extreme variation was found in the proportion of fertile to sterile eggs in a cocoon and the greatest number of worms observed to hatch was 14. There is no relation between the number of eggs and the size of the cocoon. The ova are bathed in a colourless fluid unlikely to be albumenoid (Stephenson, 1930*g*).

Development

The rate of development naturally varies with the temperature and at 12–14° C. elongation began one day after deposition. The embryos gradually elongated and by the third day they had become active in the cocoon. The worms are at first opaque and white in colour, due to the
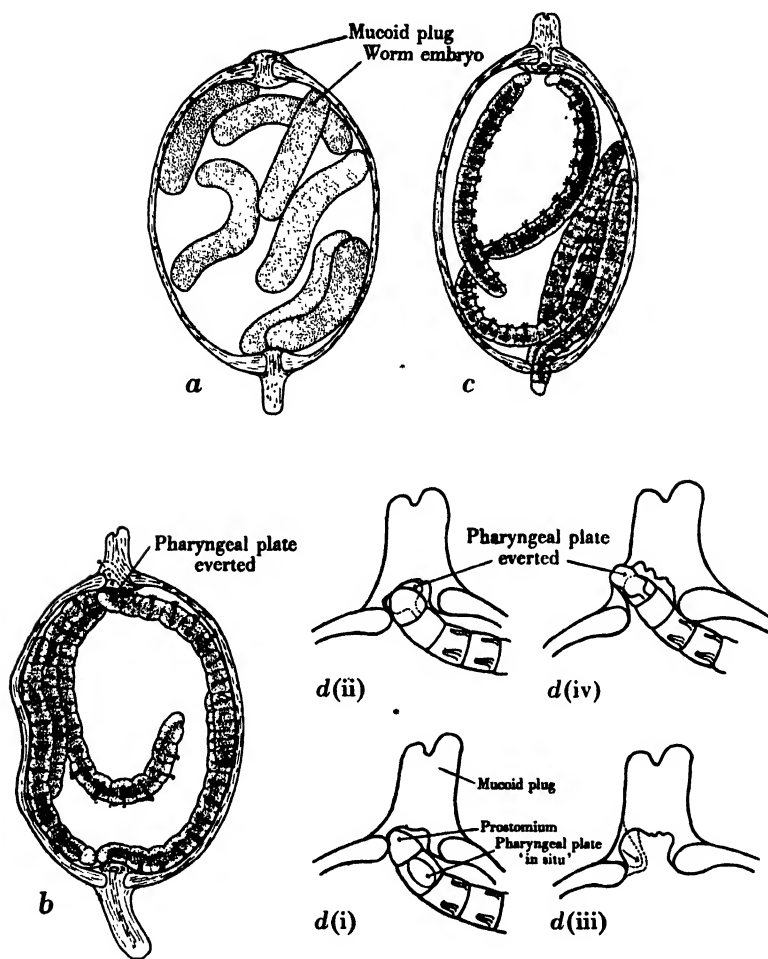
Text-fig. 1. *a*, cocoon 3 days' old at 15° C; *b*, cocoon showing pharyngeal plate everted—direction of boring indicated by arrow; *c*, cocoon showing hatching. Stages in hatching process: *d*(i), boring with prostomium before everting pharyngeal plate; *d*(ii), pharyngeal plate everted; *d*(iii), showing effect of sucking on wall; *d*(iv), showing pharyngeal plate piercing wall.

presence of yolk, but gradually become transparent as it is absorbed. Hatching at this temperature normally takes place 9–11 days after deposition. During development there is no increase in size of the cocoon.

Hatching

It was at first thought that the young worms hatched from the cocoon by lying closely against the wall and exerting an outward pressure. This same idea was also expressed by Roule (1889) who worked on an Enchytraeid which was probably a Lumbricillid. Later, however, by closely examining the cocoons it was observed that there was a definite procedure leading to hatching. This as far as the writer is aware has not previously been described. The preliminary stages consist of "nosing" with the prostomium in the region of the polar plugs, later activities being concentrated on only one of these. After one or two partial evertions the pharyngeal plate (see below) is everted over the region of the plug and the worm sucks vigorously. The suction power is considerable since the outer end of the plug quivers noticeably. The worm then proceeds to bore into the wall, again sucking with the everted pharynx. After two or three efforts by one worm it will move away and its place will be taken by another, so that an almost continuous boring and sucking is kept up. As a result, the worms gradually push and tear their way into the junction between the cocoon wall and the plug (Text-fig. 1 b, d) and as the wall in this region becomes thinner the effect of each sucking is to pull the wall inwards as shown in the diagram (Text-fig. 1d (iii)). Finally, it becomes so thin that as the pharynx is everted it pierces the wall and the worm accomplishing this crawls out to be followed by the others as they discover the rupture. The approximate time from the beginning to the end of this process is 3 hr. It is of interest that the worms attack the weakest point in the cocoon structure. This can easily be shown by placing a cover slip over a cocoon and pressing lightly, when the rupture almost always takes place at the junction of the wall and the plug.

The pharyngeal plate is a projection of the dorsal gut wall into the gut lumen. It consists of tall, columnar cells and is well supplied with muscles. Its structure and association with certain glands has been described by Stephenson (1922) who has also given a number of suggestions as to its function (1930h). It is therefore of interest to record this instance which was not mentioned by him. The worms when hatched are about 1 mm. in length comprising 13 segments, with well-developed setae numbering 2 per bundle. They are very active and commence to feed at once.

There are two interesting features about the development. In the first place, in 130 out of 200 cocoons all the eggs did not develop and in some none at all: it is probable that these eggs had not been fertilized. This was noted both in cocoons deposited under artificial and natural conditions. Secondly, during development, the active worms attacked the sterile eggs and fed upon them and these usually disappeared in a day or so, although in some cases remains of sterile eggs were present at hatching. Similar observations have been recorded for *Tubifex tubifex* (Stephenson, 1930*i*). Worms feeding in this way should be larger than those without extra provisions, so measurements were made of hatching worms and correlated with the quantity of food available as sterile eggs. Table I shows the results of the observations; the worms are divided for convenience into three groups, based on the number of sterile eggs available per 10 worms.

Table I. *Size of worms in relation to food available in the form of undeveloped eggs*

	Group 1	Group 2	Group 3
Ova available per 10 worms	0	0–5	5
Av. size of worm, sq. mm.	0·155	0·186	0·243
Length × breadth	±0·0029	±0·00307	±0·010
Range in size	0·084–0·157	0·117–0·259	0·084–0·416
No. of worms measured	62	75	31

These results show an increase in size of the worms correlated with increase of food in the form of sterile ova which is statistically significant. Lack of uniformity in the size of the worms hatching from a single cocoon was great in some cases, as the following examples show:

Cocoon 1.   0·143, 0·248 sq. mm.
Cocoon 2.   0·084, 0·127, 0·166, 0·167 sq. mm.
Cocoon 3.   0·094, 0·148, 0·173, 0·315 sq. mm.

and accounts for the wide range in size of the worms in the above groups (Table I). These observations may indicate the reason for the great variation in length found in the adult worms (9–17 mm.) and might be of importance in the study of their evolution since they seem to show the existence of intra-specific competition and are analogous to the cases of intra-uterine competition cited by Huxley (1936), in this case competition for the sterile ova replacing that for the parental food supply.

BIOTIC FACTORS—PREDACITY AND PARASITISM

Descriptions of the fauna of these beds have been given in previous papers (Lloyd, 1935, 1937; Reynoldson, 1939) and one of the interesting features is the comparative scarcity of definitive predators, especially those feeding upon the worms. It has been suggested by Lloyd (1937) that in times of food scarcity in the beds some of the dipterous larvae may become carnivorous. This is most likely to occur at off-loading periods and in the summer when the insect population is at its height. In view of the extreme difficulty of making accurate field observations, the possibility has been examined in the laboratory with a series of combinations of the dominant larvae and the worms and cocoons. A full description of the technique and results is given elsewhere (Lloyd *et al.* in the Press), but it might briefly be said that the attacks of the larvae upon the adult worms were insignificant but there is a distinct possibility that they feed readily upon the cocoons in the beds.

Nematodes were suspected of attacking the cocoons but evidence was never obtained. Their eggs have however often been found deposited on the wall of the cocoon.

Parasites of the worms and cocoons

The adult worms generally harbour a ciliate (*Anoplophryinae*) in the fore-gut, an association which seems to be of frequent occurrence (Stephenson, 1925). No pathogenic condition was evident but Stephenson has reported a case in which the alimentary canal was blocked by the ciliate and the wall degenerate. Parasites belonging to the Monocystis group of the Sporozoa invariably occurred in the segments containing the testis lobes.

Empty cocoons collected from the beds were often swarming with ciliates but these were probably attracted by the remains of the ova after hatching and are not parasites. The cocoons afforded an anchorage for Vorticellids and other peritrichous ciliates. During experimental studies of development it was noted that the cocoons were sometimes attacked and the eggs destroyed by a ciliate of the genus *Glaucoma*.

EXPERIMENTAL STUDY OF WORM BEHAVIOUR AND FECUNDITY
IN RELATION TO TEMPERATURE

I. *Temperature and worm behaviour*

Detailed investigations of the worm numbers in the surface alga showed that during periods of exceptionally cold weather the worms migrated from the surface to the warmer depths (Reynoldson, 1939).

This reaction was tested experimentally by using models of bacteria beds in one of which a moderate temperature could be maintained throughout, while in the other a graded temperature could be arranged.

Description of apparatus (Text-fig. 2). A glass cylinder 5 in. diam. and 8 in. deep was filled with clean stones taken from the bacteria beds. Small stones ($\frac{1}{4}$–$\frac{1}{2}$ in.) were used for the upper inch to increase the spread of water arranged to drip on to the surface, but the remainder were 1–3 in. in length. The cylinder rested on perforated
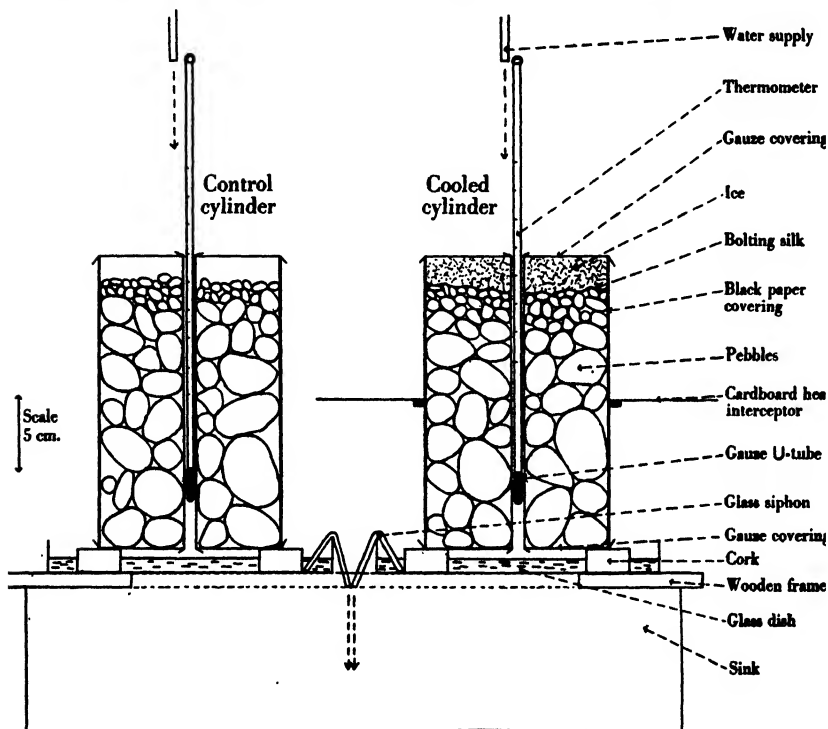
Text-fig. 2. Diagram of the model beds.

zinc, supported by three corks in a shallow circular glass dish. A siphon was arranged to empty water from this dish as it collected, conducting it to a sink. The upper surface of the glass cylinder was covered with perforated zinc to disperse further the water which was supplied as a rapid drip from a height of 6 in. above the gauze. By this means it was possible to keep the stones thoroughly wet during the experiment.

A piece of perforated zinc bent into a U-shape in transverse section, allowing a thermometer to slide up and down inside, was placed in the middle of the cylinder. It was fastened below and kept in place by the pressure of the stones packed around it. Another cylinder was arranged similarly as a control, but a space of 1 in. was left

free above the medium. Both these cylinders rested on a wooden framework over a sink.

A special thermometer, calibrated to enable temperatures to be taken at any desired depth, was used. The U-shape of the thermometer's setting ensured that the temperatures recorded were those of the stones.

In the cylinder in which the graded temperatures were desired, a layer of bolting silk was placed over the upper surface of the stones, and ice, broken into small pieces, tightly packed over it. To keep the stones as wet as in the control cylinder, it was found necessary to pipette ice-cold water over the surface every $\frac{1}{2}$ hr. The cylinders were covered with black paper to prevent any distracting influence due to lateral light.

Preliminary tests showed that 2–3 hr. after the ice had been applied the cooling effect in the lower half of the cylinder was great, whereas it was desired to simulate the conditions in the bed where the surface cools but the depths remain warmer. Therefore two lamps were arranged to throw a suitable heat on to the lower half of this cylinder, the upper half being protected by a platform of cardboard resting on corks glued to the black paper covering of the cylinder. Further tests showed that the apparatus now gave the required temperature grading.

Method. The drip of water was started some time before the beginning of the experiment so that the medium was thoroughly wet. Worms freshly collected from the bacteria bed were placed on the upper surface of the medium of both cylinders, and left to distribute themselves overnight. Next day the control cylinder was left undisturbed but the supply of water was turned off in the other and ice applied to the upper surface. Temperatures were taken every $\frac{1}{2}$ hr., in the case of the control at the top, middle and bottom levels, and in the other at quarterly "depth intervals". The experiment occupied 4–6 hr., and at the end of that time the medium from the top, middle and bottom thirds of each cylinder was removed into separate dishes and the worms collected and counted. The few worms washed out of the beds were counted in with those from the bottom third.

Table II. *Distribution of the worms and the temperatures in the two cylinders*

		Distribution of worms in % of total used				Temp. °C.		
		Top	Middle	Bottom	Worms used	Top	Middle	Bottom
Exp. I.	Cooled cylinder	12	54	34	800	1·5–5·5	5·5–8·3	8·3–9·3
	Control cylinder	68	23	9	630	11·5	11·5	11·5
Exp. II.	Cooled cylinder	39	39	23	950	2·2–6·8	6·8–8·5	8·5–10·5
	Control cylinder	43	48	9	940	13·8	13·8	13·8
Exp. III.	Cooled cylinder	19	25	54	510	2·8–6·0	6·0–8·0	8·0–8·5
	Control cylinder	47	29	26	740	11·0	11·0	11·0
Exp. IV.	Cooled cylinder	28	19	54	980	1·3–5·0	5·0–7·8	7·8–9·0
	Control cylinder	—	—	—	—	—	—	—
Exp. V.	Cooled cylinder	12	31	57	980	1·4–4·3	4·3–6·5	6·5–7·5
	Control cylinder	76	12	12	800	10·8	10·8	10·8
Av.	Cooled cylinder	22	33	45				
	Control cylinder	55	44	13				

The results are given in Table II. In the cylinder of uniform temperature, at the end of each experiment the majority of the worms was gathered in the upper half, but, in the cylinder cooled at the top, in each case the majority of the worms had migrated to the lower, warmer half, except in Exp. II where the top temperature was rather higher. This confirms the belief that the downward migration of the worms in the beds in cold weather is due to a thermotropic response.

II. *Temperature and worm fecundity*

The reduced range in daily and seasonal temperature fluctuations compared with those of the atmosphere and most natural habitats is one of the striking features of the bacteria bed, and since the breeding occurs principally in winter the relationship of temperature to worm fecundity was examined.

The experiments were carried out during the period of most active breeding of the worm in an apparatus devised by Prof. E. A. Spaul for maintaining graded temperatures. This consisted of a long zinc trough divided into compartments, one end being in an ice box and the other in a controlled warm water bath. The trough was enclosed in a case packed with felt and cork and the compartments had tightly fitting cork lids. In spite of this insulation there was a slight temperature swing of 1–2° C., the means about which are recorded in the following account.

Cocoon deposition and egg sterility. Worms were kept in the apparatus at temperatures of 1, 5, 7, 10, 15 and 20° C. approximately for a period of 4 days. The cocoons and contained eggs deposited during this time were counted and the former are expressed in Table III as a percentage of the number of worms used.

Table III. *Showing the effect of temperature on cocoon deposition and egg production*

Av. temp. ° C.	No. worms used	Total no. cocoons observed	Av. no. eggs per cocoon	% cocoons/ worms
1·5	150	0	—	0
4·5	160	17	7·0	11
7·4	149	57	6·0	38
9·8	106	54	6·0	51
15·0	128	86	7·0	67
20·2	158	106	7·0	67

The results show that increase of temperature up to 15° C. causes an increase in the rate of production of cocoons and eggs (since the average number of eggs per cocoon varies slightly), presumably due to greater physiological activity with increasing temperature. The most pronounced effect is caused by a temperature of 5–6° C.

The percentage of sterile eggs deposited at each of these temperatures has also been observed and the results indicate that a temperature of 20° C. causes an increase in sterility (Table IV).

Table IV. *Showing effect of temperature on egg sterility*

Av. temp. °C.	Total eggs observed	% sterile
4·5	560	32
7·4	588	29
9·8	522	24·5
15·0	681	26
20·2	728	42

This is not likely to be of importance to the worm in maintaining itself for two reasons; first, the worm breeds mainly in the winter, and secondly, bed temperatures of 20° C. have not been recorded over a period of 5 years' observation.

Development and hatching. With increasing temperature the rate of development increases (Text-fig. 3 and Table V), but at temperatures of 1–2° C. only 9 out of 20 cocoons showed eggs developing and of these only one hatched. These cocoons had been deposited at 7° C. owing to the difficulty of obtaining them below this temperature, and the cotton wool on which they were kept had been moistened with sewage. Here again the greatest effect within the range of viability is in the region of 5–6° C.

Two series of approximately 20 cocoons were used at each temperature gradient and it was found that for a number of cocoons the worms within failed to rupture the wall and died. The percentage failing to hatch was fairly constant (26 %) for temperatures of 7° C. and above but at 4° C. it was as high as 80 % (Table V). For these experiments tap-water had been used to moisten the cotton wool on which the cocoons had been kept, but in view of the considerable mortality resulting, boiled bacteria bed effluent was tried instead. With this the number of cocoons from which the worms failed to hatch was reduced to 3 % for temperatures of 7° C. and above, and at 4° C. was as low as 20 %. This indicates that some property of the bed effluent is more favourable to hatching than tap-water and, in addition, the time from deposition to hatching is slightly reduced. The reason for this is being investigated.

DISCUSSION

The bacteria beds of sewage works form an artificial environment to which many species of Oligochaetes, both as free-living forms and in cocoons, must constantly be carried, especially during periods of heavy

rainfall. At Leeds only three species have been able to colonize this environment successfully; *Lumbricus rubellus* Hoff. and *Lumbricillus lineatus* in the beds themselves and a species of *Nais* in the channels below. It is of interest to enquire why *L. lineatus* alone of all the small worms of this
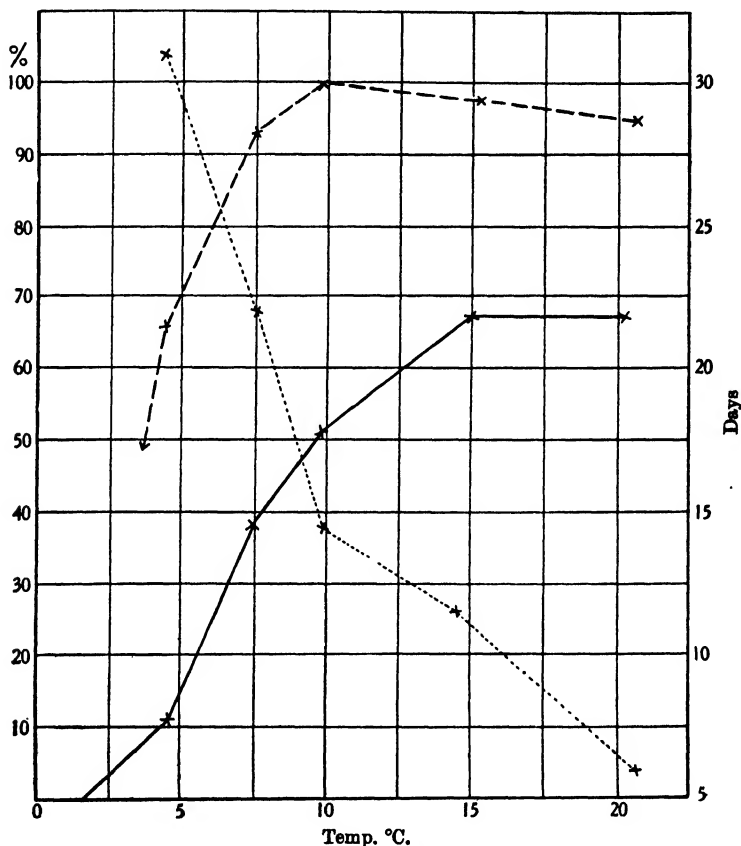
Text-fig. 3. Showing the rate of cocoon deposition development and hatching in relation to temperature, taken from Tables III & V. ——, % cocoon deposition; ————, % hatching, sewage; ·······, days to hatch, sewage.

class is able to thrive in these beds. Structurally and in its life history this worm shows no specialization which might account for it, but physiologically the worm shows a wide range of adaptability. The known facts of its distribution show that it is adaptable to a wide range of physical and chemical conditions, since it can live on the seashore or at

Table V. *Rate of development and number of cocoons hatching at different temperatures, for sewage and tap-water*

Medium	Temp. °C.	No. of cocoons observed	No. of cocoons hatching	Days to hatch	% hatching
Tap-water	4·3	13	5	41	38
	4·5	15	3	28	20
Sewage	4·4	20	16	33	80
	4·5	7	4	28	57
Tap-water	7·1	17	12	25	71
	7·4	21	16	24	76
Sewage	7·5	24	22	23	92
	7·5	19	19	21	100
Tap-water	10·0	21	13	15	62
	9·9	20	14	19	70
Sewage	9·8	23	23	16	100
	10·1	20	20	13	100
Tap-water	15·9	21	14	11	67
	15·3	22	19	9	86
Sewage	15·5	29	29	10	100
	15·0	23	22	13	96
Tap-water	20·7	24	16	7	67
Sewage	20·6	21	19	6	90
	20·3	28	28	6	100

the margins of streams. Information is needed upon the following three points before an understanding of the relationship between the worms and the beds is possible.

(1) The general resemblance which the beds bear to the normal environment of the worm.

(2) The features of this special environment favourable to the worm's existence and allowing a density of population to be reached rarely equalled in natural habitats.

(3) Factors in the special environment, structural, physical, chemical, or biotic which offer resistance to the worm's multiplication.

(1) The beds are saturated with moisture and the worms are subjected to periodic washing, though at more frequent intervals than would be experienced under natural conditions. They are exposed to fluids of rapidly varying osmotic pressure. They must be able to resist the downward flow of sewage when resting on pebbles often devoid of any growth in which they can burrow, though on the surface where the water flow is most powerful the growth of *Phormidium* (*Cyanophyceae*) provides an anchorage. The force to be resisted is less than the suction of the tide, but support, except at the surface, is less secure. There is an abundance of decaying vegetable matter for food.

(2) The structure of the beds is an important factor contributing to the worm's success. The depth prevents complete drying out when the beds are not in use—actually only the upper 4–5 in. dry out—so that the worms have a retreat against desiccation rarely found in their natural habitats where dry spells must cause a high mortality.

The temperature of the beds is much higher in winter than that of the surrounding atmosphere and most natural habitats, due to the protection afforded by the bed structure itself and the heat of the vital activities taking place within. This gives the worms a retreat from extreme cold and is favourable to a high rate of breeding, since they breed mainly in winter. It has been shown that only below 6° C. is breeding seriously affected and records show that only once has the average monthly temperature of the beds fallen below this critical figure over a period of 5 years. This must occur frequently under natural conditions.

The depth of the habitable zone affords refuge from the birds (mainly starlings and meadow pipits) which feed on the surface of the beds often in large flocks. Bird life is also abundant by streams and on the seashore but the protection is not so adequate.

Further, the bacteria beds are peculiar by reason of the comparative rarity of natural predators in the fauna. Experiments have shown that certain of the dipterous larvae living in the beds may feed upon the cocoons in time of food shortage but, nevertheless, it is reasonable to say that here predators are very few, enabling the worms to increase almost unchecked by such a depleting factor.

(3) Factors offering resistance to the worm's multiplication are relatively few in the bacteria bed. The osmotic variations of the sewage are no greater than those occurring on the seashore and cannot be regarded as an unfavourable factor, but the peculiarities of the chemical composition of the sewage need to be tolerated. The sewage at Leeds is not characterized by any special trade waste nor is it subject to any drastic chemical treatment, and it is, therefore, more suited to the worm which can evidently tolerate a wide range. Worms are much less numerous at Huddersfield where drastic chemical treatment is carried out, and trade wastes from dye works form a large part of the sewage.

Sewage is characterized by a lack of dissolved oxygen and it is a general feature of Invertebrates that those with haemoglobin in the blood more frequently inhabit places where the oxygen tension is low. It has been shown (Leitch, 1916) that *Planorbis* and *Chironomus* larvae (species not stated) utilize the oxygen binding properties of the haemo-

globin to enable them to live at the low oxygen tensions of their habitats. Friend (1916) has suggested that the possession of haemoglobin by some Enchytraeids has enabled them to inhabit polluted areas. Observations in the present case indicate that haemoglobin is unnecessary in the bacteria beds, since although the sewage delivered on to the beds is usually devoid of dissolved oxygen, the worms are only covered by a thin film for most of the time and breathe atmospheric oxygen which is abundantly supplied by the aeration of the beds. The haemoglobin might help worms to pass through the various regions of the sewage plant to the beds where they are immersed.

Reference has been made to the need for resistance by the worms and cocoons to the flow of sewage. The worms avoid being washed down by their activity and the cocoons are firmly attached to the pebbles. This is of interest since it is only in the genus *Lumbricillus* of the Enchytraeids that the cocoons are recorded as being attached to the substratum (Stephenson, 1930*i*).

This account shows clearly the unique type of environment formed by the bacteria bed with regard to the Enchytraeid worms and *L. lineatus* in particular. It is a habitat which encompasses the main features of the wide range of habitats of this worm in nature, especially its amphibious character, the presence of a water flow, the wide range of chemical conditions and an abundance of suitable food. In addition it possesses definitely favourable features to the worm's existence, such as a higher temperature in winter and a scarcity of predators. Not only does it favour the worm in this direct way but also in an indirect manner, for in the complex of factors are those which inhibit colonization by other Enchytraeids (the nature of the habitat naturally inhibits purely aquatic types such as *Nais*), the most efficacious of which is probably the peculiar chemical conditions which few are capable of withstanding. Thus, this worm by virtue of its great tolerance can multiply unchecked by competition with related species in this artificial environment and take full advantage of the favourable conditions which bacteria beds offer.

SUMMARY

1. A survey of the natural and artificial habitats of *Lumbricillus lineatus* Mull. shows a wide range and toleration of diverse chemical conditions.

2. The bacteria beds are described in relation to the needs of the worm.

3. An account is given of the life-history of the worm including breeding season, copulation, cocoon deposition, development and hatching. The latter is given in detail since it is believed to be new. It demonstrates a use of the eversible pharyngeal plate. Experiments show that the hatching worms increase in size with an increase in quantity of food available within the cocoon in the form of undeveloped ova.

4. A short account of the parasites and possible predators is given.

5. Experiment shows that the worms migrate from low temperatures to higher, a reaction observed in the beds during cold weather.

6. Temperatures below 6° C. affect adversely: (*a*) cocoon deposition, (*b*) rate of development, (*c*) hatching. The beds, in contrast to natural habitats, rarely fall below 6° C. Egg sterility is increased at a temperature of 20° C.

7. The bacteria beds form an environment for this worm which includes all the main features of its natural habitats. The higher temperature in winter, the protection from desiccation and birds, and the scarcity of natural predators allow excessive multiplication of the worm. Further, certain factors in the beds, probably the peculiar chemical conditions, prevent colonization by other similar worms so that competition with other Oligochaetes is at a minimum. Thus, by virtue of its great degree of physiological adaptability, this worm is able to thrive in the bacteria beds to an extent not found in any natural habitat.

REFERENCES

FRIEND, H. (1916). The bionomics of English Oligochaeta. II. British Enchytraeids. The role of *Pachydrilus*. *Sci. Progr.* 11, 12.

HUXLEY, J. (1936). Natural selection and evolutionary progress. *Nature, Lond.*, 138, 451.

LEITCH, I. (1916). The function of haemoglobin in invertebrates with special reference to *Planorbis* and *Chironomus* larvae. *J. Physiol.* 50, 370.

LLOYD, LL. (1935). The bacteria beds of sewage works as an environment for insects. *Proc. R. ent. Soc. Lond.* 10, 34.

—— (1937). Observations on sewage flies; their seasonal incidence and abundance. *Proc. Inst. Sewage Purif.* Part 1, 1.

Fig. 1.
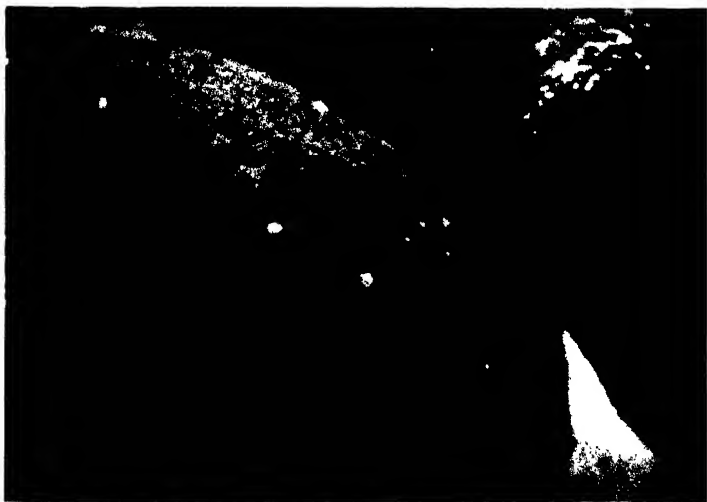
Fig. 2.

REYNOLDSON.—On the Life-History and Ecology of *Lumbricillus lineatus* Müll. (Oligochaeta) (pp. 782–799)

LLOYD, LL., GRAHAM, J. F. & REYNOLDSON, T. B. Materials for study in animal competition. *Ann. appl. Biol.* (in the Press).

REYNOLDSON, T. B. (1939). Enchytraeid worms and the bacteria bed method of sewage treatment. *Ann. appl. Biol.* **26**, 138.

ROULE, L. (1889). Étude sur le Développement des Annélides, et en particulier, d'un Oligochaete Limicole Marin (Enchytraeoides Marioni). *Ann. Sci. nat. Zool.* Ser. 7, **7**, 107.

SOUTHERN, R. (1909). A contribution towards a monograph of the British and Irish Oligochaeta. *Proc. R. Irish Acad.* **27**, 8.

STEPHENSON, J. (1922). On the septal glands and pharyngeal glands (Oligochaeta) of the Microdrili. *Trans. R. Soc. Edinb.* **53**, 241.

—— (1925). The Spitsbergen and Bear Island Expedition. Oligochaeta. *Proc. zool. Soc. Lond.*, p. 1294.

—— (1930). *The Oligochaeta.* (a) p. 609; (b) p. 616; (c) p. 617; (d) p. 436; (e) p. 437; (f) p. 472; (g) p. 479; (h) p. 77; (i) p. 498; (j) p. 475. Oxford Univ. Press.

WELCH, P. S. (1914). Studies on the Enchytraeidae of North America. *Bull. Ill. Lab. nat. Hist.* no. 10.

EXPLANATION OF PLATE XLIII

Fig. 1. A characteristic clump of worms on a stone beginning to break up—the white patches visible on some worms indicate the segments containing testes and ovaries. × 2·5 approx.

Fig. 2. Photograph of stone from beds showing cocoons attached. × 3·0 approx.

STUDY OF *BYSSOCHLAMYS FULVA* AND CONTROL MEASURES IN PROCESSED FRUITS

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(With 2 Text-figures)

CONTENTS

I. INTRODUCTION

THE principle underlying the preservation of fruit in cans or bottles is to seal the raw fruit immersed in a sugar solution in the container, and sterilize it by heat. Experience has shown that, as a rule, moulds and yeasts can be destroyed by heating the cans for a time which gives a palatable product of attractive appearance, and heat resistant bacteria are rendered innocuous because of their inability to grow in acid media such as fruit juices. Spoilage of preserved fruit by micro-biological activity has been of comparatively rare occurrence and could usually be traced either to mistakes in the routine of heating, or to micro-organisms entering the container after sterilization, owing to unsatisfactory sealing. When such spoilage has occurred it has been accompanied by gas production, leading to "blowing" or bulging of the ends of the container, and so the trouble could be detected by the external appearance of the can.

A form of spoilage caused by a mould with heat resistant ascospores, *Byssochlamys fulva*, Olliver & Smith (1933), has been encountered in recent times and has been described by Hirst & McMaster (1933) and by Olliver & Rendle (1934). In affected cans the texture of the fruit is very soft, and any shaking of the container, such as happens in transport, causes the fruit tissues to break down and become a pulpy mass. Disintegration may even take place while the containers are in the store. As a rule there is not sufficient gas production in the container to blow out the ends, so that affected cans are unrecognizable until they are opened. This is of commercial importance, since such cans may reach the consumer and cause prejudice against the products of the manufacturer concerned and even against canned goods in general.

Information about the physiology of *B. fulva* is given by Olliver & Rendle (1934), who state that it is a saprophyte which is easily cultured on media and grows especially well on fruit juices of acid reaction and of approximately 10% sugar content. It is unable to attack living fruit tissues but disintegrates those of preserved fruits. The presence of the fungus in the processed contents is due to the high heat resistance of the ascospores. The original infection is probably introduced into the can on the fruit, as ascospores were detected on raw fruit in orchards and in the factory by heating the material before plating it out; in this way mould spores were killed but *B. fulva* ascospores remained viable. Olliver & Rendle's experiments showed that a suspension of ascospores in fruit syrup withstands a temperature of 86–88° C. for 30 min., and when the suspension is heated at a similar rate to that at which the contents of a can are heated during processing, viable spores are present until a temperature of 98° C. is reached. This temperature is considerably higher than that attained during normal processing of fruit.

Hirst & McMaster (1933) gave the history of the occurrence of the trouble as experienced at the Fruit and Vegetable Preservation Research Station, Campden. They first encountered it in 1931, but did not associate it with *B. fulva*; a small disk of mycelium was often seen amongst the disintegrated contents of the can, but it would not grow in culture and appeared to be dead.

In subsequent years other instances were reported by canners from most parts of the country so that the trouble appeared to be widespread. On the other hand observations at the Research Station suggested that severe fruit infection might be confined to certain orchards. For two consecutive years all the fruit (strawberries) from a certain grower gave a badly infected product both at the Research Station and at a nearby

factory, whereas similarly processed fruit from other sources behaved satisfactorily. This observation suggested that heavy infection might be confined to certain orchards, so that it was desirable to investigate the occurrence of the fungus in the field. Also, from the growers' point of view, it was desirable to know more about the field occurrence, as those growers whose fruit had once given an infected product had difficulty afterwards in selling their fruit to canners.

Hirst & McMaster (1933) described experiments in which inoculated cans were processed for various times, and which suggested that the ascospores might be killed during processing in some of the hard fruits, but not in soft fruits. Even in the hard fruits, where the mould was killed, the temperature attained in the can was only between 87–91° C., which was considerably below that (98° C.) at which Olliver & Rendle had found the spores to remain viable in their experiments. Thus it was possible that there was some factor which made sterilization in the can easier than was indicated by laboratory results.

The work described in this paper was undertaken to determine to what extent the fungus was disseminated in the field, whether any field control was practicable, and to what extent control was possible during the normal canning process. Progress reports containing some of the results included in this paper were published during the investigation (Hull, 1934, 1935).

II. Occurrence in the field

A method of isolation which took advantage of the heat resistance of the ascospores of *B. fulva* was used to determine whether spores were present on material from the field. Samples of leaves, fruit, straw, etc. were collected in plugged sterile tubes of appropriate size. These were poured in the laboratory with hot (*c.* 60° C.) potato-sucrose-agar, acidified with hydrochloric acid till it would just set, and while the agar was still liquid the tubes were heated in a water-bath at 80° C. for 30 min., sloped, and incubated at 30° C. when they had set. This procedure was found by experiment to give the maximum count of samples showing *B. fulva*; shorter heatings yielded tubes with many other micro-organisms and these prevented *B. fulva* from growing.

Samples were collected and treated in this way from a strawberry plantation at Campden the fruit of which had given an infected "pack" in the previous year. A few samples of dead leaves were taken in January, · 1933, and some positive results were obtained. In April both young and

old leaves collected in the field yielded the fungus in 33 of the 100 samples taken and numerous subsequent samplings from the same plantation gave similar results. Ripe berries sampled in the field in the middle of July showed 26 % contamination.

Strawberry plantations in Kent were visited in June and large numbers of leaf samples were collected. Contamination varied somewhat in different districts. Thus two fields near Maidstone showed 6 and 13 % respectively; two fields at Barming showed none in 40 samples taken from each; fields at Yalding and Goudhurst gave 25 and 20 % respectively; four fields near Faversham each gave 5–8 %, and one field at Swanley showed 7 %. Samples of the fruit which had been obtained from these plantations were collected at the canning factories and showed the presence of *B. fulva* spores. Samples of leaves taken in April from various fruit trees in orchards near Colchester were found to be slightly contaminated. In certain plantations in Gloucestershire of raspberries, loganberries, black-currants, gooseberries and plums, fruit and leaf samples were collected at various times, and contamination was found in all cases. Samples of fruit, usually 50–100 berries, were collected throughout the season on arrival at the Research Station, Campden, at factories and in orchards, and varying amounts of contamination were found. Further reference to the extent of contamination of the fruits is made in Table X.

No fungal growth was observed on the samples as collected, and the fact that growth of *B. fulva* usually started from one centre suggested that occasional ascospores had lodged on the sample. Evidence bearing on the source of these spores was obtained from the following observations. Runners from strawberry plants in the fields at Campden had been planted during the previous year in an isolated field about 10 miles away. There were no other orchards within half a mile of this field, the surroundings being pasture and plough-land. Samples of leaves taken from here at various times showed 5–10 % contamination as compared with 15–20 % for runners taken from the same stock and planted near the original field at Campden. This suggested that there was some source in the Campden district which was emitting spores into the air near the fruit plantations.

It was found that mummified plums, taken in the winter from an orchard close to the strawberry fields, were heavily contaminated with *B. fulva* ascospores. In cultures prepared from such plums, fungal growth began at numerous centres in the agar slope, whereas, in similar preparations from leaves, growth usually took place from one centre only.

It was suggested that the ascospores had been produced on the mummified plums and from them had reached the leaves.[1]

Field evidence in support of this view was obtained in a raspberry plantation at Evesham, in which the canes were planted under plum trees. In one half of this orchard the trees were old and carried numerous mummified plums, in the other the trees were young and had few mummified plums. Leaves from raspberry plants in the two halves showed 34 and 18 % contamination respectively.

Fruit refuse, such as "second crop" raspberries which had been left on the canes, was found in some cases to be heavily contaminated after the manner of mummified plums.

A secondary source of contamination was established in one case where old chip baskets which had been used the previous season were being used again after storage. Remains of fruit present on the linings were heavily contaminated with *B. fulva* ascospores, which had been produced *in situ*.

It was confirmed that *B. fulva* does not parasitize living fruit tissues. Fruits killed by heating at 100° C. for a few minutes supported prolific and rapid growth when incubated at 25° C. Attempts were made to grow *B. fulva* on fruits which had been overrun by parasitic fungi, but with only slight success. Dry mummified plums do not support growth, but in some cases slight growth was obtained when they were moistened, although often the growth of other fungi predominated and that of *B. fulva* could not be detected. *B. fulva* grew very prolifically on autoclaved mummified plums.

Samples of straw taken from a strawberry field a few days after it had been laid showed slight contamination but less than that obtained from leaf samples gathered at the same time; thus it was not likely that infection was brought into the field on the straw.

No support was found for the suggestion of Olliver & Rendle (1934) that the fungus might be a soil organism. It did not grow on sterilized or unsterilized soil. Fruits picked from the ground showed no greater contamination with *B. fulva* than others gathered from the trees above.

The foregoing observations indicate that the natural substrate of the fungus is fruit refuse, more particularly mummified plums. In view of the high temperature at which the fungus grows and produces asci it may be assumed that growth and ascus formation takes place during the

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[1] Attempts to demonstrate microscopically the presence of mycelium or ascospores of *B. fulva* in surface scrapings of mummified plums were unsuccessful, possibly because of the large amount of fungal spores (*Monilia*, etc.) and debris present.

summer months when fruit refuse is abundant, and that later the asco-spores are scattered indiscriminately over the neighbouring vegetation by air dissemination.

III. Physiological characteristics

(a) Methods and materials

Throughout the investigation frequent isolations were made from affected cans and bottles of fruit, and when the fungus developed it was invariably found to be in pure culture. Occasionally no growth resulted but the presence of the fungus could be confirmed by finding hyphae or a disk of mycelium amongst the disintegrated contents. Cans inoculated with ascospores of the fungus and subjected to the normal factory process showed the characteristic symptoms of disintegration of the fruit tissues. Reisolation gave pure cultures of the fungus; this was repeated frequently and left no doubt that the fungus was the cause of the spoilage.

Isolations were made from a suspected can or bottle by withdrawing a 5–10 c.c. sample under aseptic conditions and placing it in a sterile Petri dish where it was mixed with either nutrient or plain agar, or in a tube, in which it was incubated in the liquid state. If the fungus was present growth became visible within 36 hr. at 30° C. and its identity could be recognized by the characteristic buff-coloured conidial structure. For confirmation, the cultures were occasionally kept longer until asci were produced.

Freshly isolated cultures produce asci readily on potato-dextrose-agar and other plant-extract media, but less profusely on the usual synthetic media. At 30° C. the beginnings of ascus formation are visible in 4–5 days and mature ascospores are present after about 10 days. At 25, 20 and 15° C. ascospores could be found on plates after 8, 19 and 42 days respectively. The rate of formation of ascospores is, therefore, relatively slow at ordinary summer temperatures so that there is no risk of their increasing greatly in number during the time the fruit is being handled. Although an isolate gave satisfactory ascus production at first, after subculturing several times during 4 months it gave colonies which were covered with a snow-white fluffy mycelium. This became a tough mass on the surface of the agar and produced very few or no ascospores, so that new cultures for ascospore production had then to be obtained from affected cans.

The asci could easily be picked up in masses from the surface of the agar with a needle, and they produced a uniform suspension when shaken with water. As the ascus usually remained intact even after the suspension had been filtered, centrifuged and washed, it was the germination of the ascospores while still in the ascus which was studied and all the counts mentioned later deal with asci and not ascospores. On germination one or more ascospores develop a swelling at one end, as large as the spore itself, from which one or more germ tubes grow. The ascospores remain together after germination so that an ascus with germinated spores appears large and is thus easily identified, even if the asci are somewhat clustered together.

Germination studies were carried out by spreading a suspension of asci of suitable density over the surface of plain or nutrient agar plates, acidified with 0·5% malic acid, and incubating at 30° C.

Determinations of the thermal resistance of spores were made by adding a dense suspension of asci, prepared by filtering cultures through muslin and centrifuging, to 10–20 volumes of water heated to the desired temperature in a thermostatically controlled water-bath. Samples were then removed from time to time for germination studies.

(b) *Thermal relations of spores*

Unheated ascospores when placed on agar either do not germinate or do so sporadically; after heating to a certain degree, quick and regular germination is obtained. A similar phenomenon was reported by Gwynne-Vaughan & Williamson (1930) when young ascospores of *Humaria granulata* failed to germinate unless heated. The effect on the germination of asci of *B. fulva* of heating in distilled water at temperatures between 65 and 85° C. is shown in Table I.

Table I. *Percentage germination of asci after heating*

% germination after four intervals (hr.)

Temp. and time of heating	14	21	25·5	38
85° C. 10 min.	0	0	+	38
30 ,,	0	0	0	1
80° C. 10 min.	+	96	95	Weft
30 ,,	+	70	72	,,
75° C. 10 min.	+	99	98	Weft
30 ,,	+	93	96	,,
70° C. 10 min.	+	28	41	Weft
30 ,,	+	49	51	,,
65° C. 10 min.	0	0	0	5
30 ,,	0	0	2	3
Unheated	0	0	0	<1

+ Germination just beginning.

It is clear from Table I that optimum germination is obtained by heating the spores for 10 min. at 75–80° C. or for 30 min. at 75° C.

Fig. 1 shows the effect on germination of various periods of heating at 70 and 85° C.

Heating at 70° C. stimulated germination, and the effect was correspondingly greater the longer the time of heating, up to the maximum used in the experiment. This curve represents the germination which had taken place after 15 hr. incubation. With further incubation the percentage germination increased on all plates and was probably potentially 100 % in all cases, though this could not be observed in practice as the mycelial growth from the quickly germinating spores smothered the plates. At 85° C. a short exposure (*c.* 1 min.) gave 100 % germination but the percentage germination decreased rapidly with longer exposures. A large proportion, approximately 90 % of the asci,

were killed relatively easily, but many of the last 10% had a much greater heat resistance.
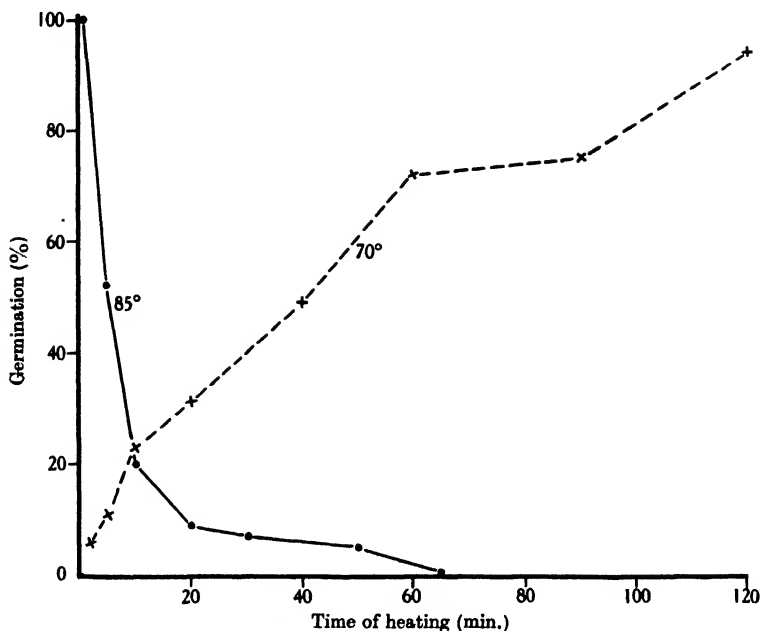
Fig. 1. Percentage germination of spores after being heated for various times at 70 and 85° C.

Determinations of the lengths of exposure which were necessary at various temperatures for killing all spores gave somewhat irregular results so that the figures obtained from any one experiment cannot be relied upon. Table II gives the *maximum* time of resistance in distilled water for the given temperature, as obtained in a number of experiments.

Table II. *Heat resistance of spores*

Temp. of heating (° C.)	94	92	90	88	86
Time above which no growth was obtained (min.)	2	5	20	40	100

A comparison of Table II with Fig. 1 shows that while the majority of spores are killed by a short exposure to 85° C., long times are required at even higher temperatures to ensure complete sterilization. An important conclusion derived from these experiments is that a small fall in temperature necessitates a great increase in the time of heating necessary to kill *all* the spores.

Henderson Smith (1923), working with spores of *Botrytis cinerea*, found that the curve representing the percentage of spores surviving various times of heating between 37–50·3° C. is of sigmoid nature. The amount of variation of heat resistance between individual spores is less than that of *Byssochlamys fulva* ascospores, but as with *B. fulva* the temperature coefficients for the reaction of killing *Botrytis* spores by heat are very high.

The heat resistance of *Byssochlamys fulva* ascospores was also determined under conditions of gradually increasing temperature. A heavy suspension of ascospores in distilled water was heated in a water-bath and samples were pipetted out at intervals and placed in "medical flats" containing apple extract solution. The temperature of the suspension when the sample was removed, and the time taken to attain that temperature, were recorded. The medical flats were incubated for 4 days and then the dry weight of the mycelium which developed, which was a function of the number of spores surviving, was determined. The results are shown in Table III.

Table III. *Growth obtained from heated ascospores*

Time of heating min.	Temp. ° C.	Mycelium weight g.
4	85·0	0·275
5	89·5	0·282
6	92·5	0·203
7	94·0	0·009
8	95·0	0·001
9	96·0	Nil

Thus, heating above 89·5° C. reduced the amount of mycelium which was produced, and none was obtained after heating to 96° C., even when the samples were incubated for a longer period. At 96° C. all the spores were killed, so this temperature is the approximate thermal death point of the ascospores, although the value would vary somewhat according to the rate at which the temperature was raised.

Heat resistance of different isolates. Ascospores from different isolates behave differently on heating. Cultures isolated from different sources were grown under similar conditions and ascus suspensions from each were heated for various times at 85° C. Table IV gives the percentage germination after 10 min. heating. Confirmatory data were obtained for other periods of heating.

Age of culture and heat resistance. Ascospores of ages varying from 3½ to 30 weeks were obtained from cultures which had been maintained at 30° C. The cultures were isolated from infected cans which had been

inoculated with the same stock culture. Suspensions of these ascospores when similarly heated gave germination figures ranging from 2 to 69%, but this variation was not obviously correlated with the ages of the cultures.

Table IV. *Percentage germination of asci after heating*

| | % germination Time of incubation (hr.) | |
Origin of culture	26	45
From a can; subcultured for some time	2	38
From orchard, Campden	11	32
From infected strawberries canned at Evesham	30	Weft
From infected strawberries canned at Whitchurch	54	Weft
From field, Evesham	91	Weft
From field, Kent	0	0
From field, Colchester	84	Weft

Effect of reaction of heating medium on heat resistance. The heat resistance of spores is influenced to some extent by the reaction of the medium in which they are heated. Fig. 2 gives the average results of several experiments in which spores were heated in buffer solutions covering a range of pH values.
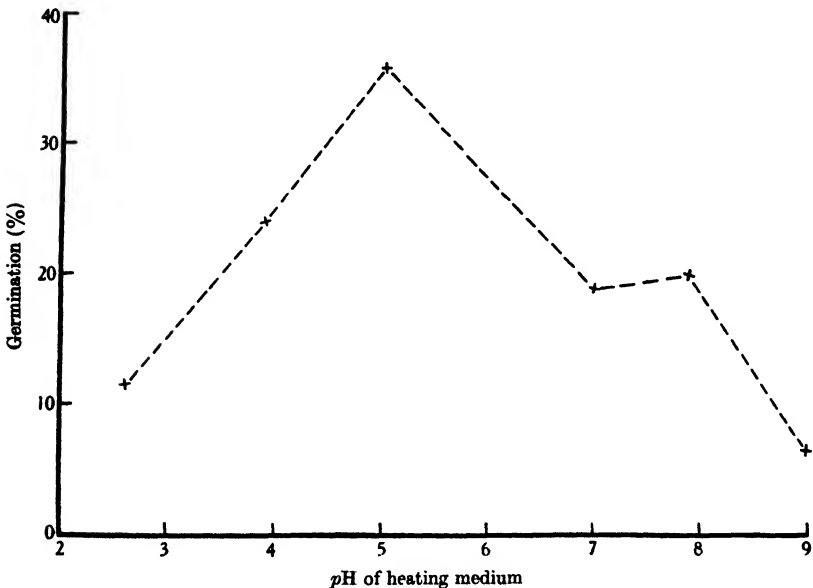

Fig. 2. Percentage germination of spores heated in solutions of various pH values.

Heat resistance was greatest at about pH 5. It is interesting to note that in the canning process the pH of the syrup taken at the middle of the "cook" varies from pH 2·9 in loganberries to pH 3·8 in strawberries (Adam, 1934).

Effect of concentration of sucrose in heating medium on heat resistance. ·The concentration of sucrose in the solution in which asci were suspended had a considerable effect on the heat resistance of the asci. Table V shows the percentage germination, after 40 hr. incubation, of asci which have been treated in various concentrations of sucrose and then placed on plates of plain agar.

Table V. *Percentage germination of asci heated in sucrose solutions*

	% germination Time of heating at 84–5° C. (min.)			
% sucrose	5	10	20	30
0	Weft	65	21	1
5	,,	62	5	1
10	,,	77	16	11
30	,,	Weft	37	15
65	,,	,,	Weft	75

Increasing the sugar concentration had a protective action on the asci, rendering them more resistant to heat treatment. In this respect Baumgartner & Wallace (1934) found that sucrose concentrations between 10 and 30 % had a similar protective action on vegetative cells of *B. coli*, but up to 50 % had no effect on bacterial spores or strains of *Torula*.

Heat resistance and germination of conidia. Whereas ascospores showed a remarkable heat resistance, conidia were killed relatively easily. All the spores in a suspension of conidia were killed after heating for 10 min. at 70° C.: heating for 15 min. at 51·5° C. reduced the germination to under 10 % but the remainder were not killed after 30 min. at this temperature. Unheated conidia germinated in apple extract at 37° C. within 4 hr. and were reproducing conidia by this time.

(c) *Factors influencing ascospore germination and mycelial growth*

Temperature. The following data illustrate the effect of the temperature of incubation on the germination of pre-heated (75° C. for 10 min.) ascospores. At 38 and 32° C. germination began after 10 and 15 hr. respectively, the majority of the asci germinating simultaneously and the remainder shortly afterwards. At 25 and 20° C. germination had begun within 24 and 43 hr. respectively, but ungerminated asci were

seen in the tangle of mycelium long after this. At 15° C. there was no germination in 87 hr.

Radial growth on potato-dextrose-agar was fairly rapid at 35° C., amounting to 3·6 cm. in 3 days. With lower temperatures it diminished progressively and at 15° C. was very slow. At 8° C. there was no growth.

The dry weight of mycelium produced in Richards' solution after 5 days' incubation at various temperatures is shown in Table VI.

Table VI. *Dry weight of mycelium at various temperatures*

Incubation temp. (°C.) ...	20	26	28	32	41
Mycelium dry wt. (g.)	0·140	0·436	0·648	0·999	0·400

The optimum temperature for ascospore germination and mycelial growth thus lies in the neighbourhood of 35° C.

Reaction of medium. Ascospores were placed on plates of potato-extract-agar adjusted to pH values between 1·8 and 8·0. The ascospores failed to germinate at pH 1·8 but germinated on all the other plates; the most rapid germination and growth took place at pH 3·5. This is close to the value (c. pH 5) at which the maximum heat resistarce of ascospores was obtained.

Sucrose concentration of medium. Both ascospore germination and mycelium growth took place in high sucrose concentrations. Asci, previously heated to 75° C. for 20 min. to ensure even germination, were placed on agar plates containing various concentrations of sucrose up to 70%. Germination was observed within 18 hr. on the plates having sucrose concentrations up to 50%, and on the remainder after 37 hr. It was more rapid on the 20% than either the 0 or the 40% plates. All plates eventually showed 100% germination, so that the main effect of sucrose concentration greater than 20% was to delay germination. In the higher concentrations of sugar the germ tubes of the spores were numerous and fine.

When heated spores were grown in apple extract solution containing added sucrose up to 65%, diminished weight of mycelium was obtained with each successively higher sugar concentration, but even at an added concentration of 65% growth still occurred. The sucrose concentrations in cans of fruit are usually about 30%, and these would not cause any pronounced depression of mycelial growth.

Antiseptics. The asci showed remarkable resistance to antiseptics. A suspension of spores was immersed in solutions of various antiseptics, centrifuged after a given time and the spores washed in sterile distilled water. Growth was obtained after immersion in 10% formaldehyde for

10 min., in 10 % "Lysol" for 30 min., in 0·5 % sodium hypochlorite for 10 min. and in 0·5 % mercuric chloride for 36 min.

Carbon dioxide concentration. In a later section the growth of the fungus in concentrations of carbon dioxide of its own production up to 65 % will be described. In an experiment in which ascospores were placed on plates of potato-sucrose-agar and in apple extract in atmospheres with different concentrations of carbon dioxide, diminished growth and much retarded germination were obtained at concentrations above 60 %. The fungus is thus relatively insensitive to carbon dioxide.

Desiccation. Ascospores are not easily killed by desiccation, as tube cultures which had been kept in a dried condition for 4 years produced growth on subculturing.

(d) *Enzyme production*

Growth is accompanied by the production of a pectin-destroying enzyme, which causes the breakdown of the fruit tissues. The enzyme was detected by the softening of potato disks in solutions in which the fungus had been growing, but the action was slow. Concentrated enzyme extracts were prepared by germinating ascospores in medical "flats" at 30° C., collecting the resulting mycelium while very young and following the usual method of enzyme extraction (Brown, 1915). The enzyme extract thus obtained rapidly disintegrated potato disks and the tissues of canned fruits.

IV. Canning experiments

(a) *Methods and materials*

Various modifications of the fundamental canning process are used in practice. Briefly, the process is as follows. The fruit is prepared and filled into the cans and is covered with a warm sugar solution to within about ¼ in. of the top of the container. The cans are then placed in hot water to remove air trapped amongst the fruits; this is called the "exhaust" process. The lids are seamed on and the cans placed in water, usually at boiling point, for the "cooking" process. After the appropriate time the cans are removed and cooled. As the lids are sealed on when the contents are hot, the internal pressure is reduced on cooling and the reduction below atmospheric pressure is called the "vacuum".

In all the inoculation experiments in cans performed at Campden the procedure was standardized as much as possible. The cans used were of size A 2. The syrup had a density of 45° Brix[1] unless otherwise stated; it was filled at 170° F.[1] by hand. The cans were exhausted in a bath at 185° F. for 6 min. and the "cooks" were made in

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[1] As in canneries, the Brix hydrometer was used for measuring syrup densities. It gives a direct reading of the percentage of sugar by weight in the solution. The Fahrenheit temperature scale is also employed in canneries and so is used in this section.

boiling water. Where possible the cans were kept stationary in the cooking bath, but in some of the soft fruits a sufficiently high internal temperature could not be attained by this method, so the cans were laid on their sides in wire trays and rolled gently at 2 min. intervals. This method is referred to as a "rolling cook" in contrast to the former which is called a "stationary cook". A "rotary cook" refers to the mechanical process normally used in factories.

Inoculations of three densities of spore suspension were used. A spore suspension was made in distilled water from a culture about 3 months old. The suspension was filtered through muslin and centrifuged. To each 0·1 c.c. of centrifuged spores, 10 c.c. of sterile water were added, and the spores were shaken into an even suspension. This constituted the "heavy inoculum" used in the experiments, and 1 c.c. of this suspension was added to each can. The "standard inoculum" was this suspension diluted × 10, and the "light inoculum" was the "heavy inoculum" diluted × 1000. A count of the "light inoculum" showed the presence of approximately 100 spores/c.c., but owing to the method of making the spore suspension, this figure was not taken as absolute, but rather as showing the order of the number of spores. Thus, there would be approximately 10^5 spores in the cans with heavy inoculum, 10^4 with standard and 10^2 with light inoculum. In all cases the cans were inoculated after the fruit had been inserted but before adding the syrup.

The fruit used in the experiments was obtained from local growers. While it was being picked and placed in the cans, samples were also placed in sterile tubes by the pickers, and were examined by the method already described for the presence of ascospores of *Byssochlamys fulva*. Any inoculum added to a can was additional to that naturally introduced on the fruit. The fruit in control cans contained the natural infection only.

After processing, the cans were stored at room temperature or at 37° C. if they were to be examined within a fortnight. In any case they were always given a period of 3–7 days at 37° C. before examination. They were opened under aseptic conditions and a sample of their contents examined for the presence of *B. fulva*. In each case two samples (approx. 5 c.c. each) were placed in Petri dishes and poured with agar, and one tube sample (approx. 10 c.c.) was kept. The texture of the fruit was noted so that had the fungus grown and subsequently died a deceptive result would not be obtained.

Two methods of measuring the internal temperature of the contents of a can during the cooking process were used. When the cans were heated in a stationary tank lids pierced with a large hole were used, and after the lids had been seamed on, a thermometer passed through a rubber stopper was inserted tightly in the hole. The length was such that the bulb reached to the centre of the can. The can was then placed in the boiling tank and the rise in temperature at the centre of the contents recorded. When the cans were rolled or extracted from a rotary cooker, the piercing armoured thermometer was used. This had a base armoured with a pointed metal case which was plunged into the end of the can to a depth defined by the position of a stopper on its stem. The thermometer had a considerable heat capacity, so before use it was heated approximately to the temperature expected to be recorded.

Although the results obtained on different cans with one instrument were comparable, those obtained with the different instruments were not. This was because the fixed thermometer was inserted in the syrup between the fruits, whereas the piercing thermometer took an average record of the temperature of the syrup and that of any

fruit which it happened to pierce. Also, syrup often spurted out of the can when the end was pierced with the thermometer, and this would involve some movement of the contents which normally would not take place. However, as the instrument was used mainly for recording temperatures in agitated cans, the error caused by this spurting was not so serious as it would have been in the case of stationary cans.

The general results obtained were that a can agitated in the cooking bath heated much more quickly than a stationary one, and that with a stronger syrup the contents heated more slowly than with a weaker syrup. Apart from these variations with any one fruit, soft fruits such as strawberries and raspberries heated much more slowly than smooth hard fruits such as gooseberries and plums.

(b) *Experimental results*

Inoculations with standard suspension of ascospores. Cans of golden plums were inoculated with the *standard* suspension and cooked for various times. The amount of infection obtained is shown in Table VII.

Table VII. *Infection in inoculated cans of golden plums*

Time of "cook" (min.) ...	12	16	20	24
Internal temperature (°F.) ...	181·5	189·3	191·5	193·3
Proportion of cans infected* ...	5/5	4/5	0/5	0/5

* The numerator shows the number of cans infected and the denominator the number of cans used.

When the time of "cook" was longer, and consequently the internal temperature higher, the proportion of infected cans was reduced. This result is typical of a series of experiments with various fruits; the details of the times of "cook" necessary to produce sterility in all the cans used and the internal temperature recorded for that time are given in Table VIII.

Table VIII. *Sterility in inoculated cans produced by heat treatment*

	Time of "cook" min.	Internal temp. °F.
	Stationary	
Black currants	20	195·2
Victoria plums	24	188·0
Damsons	20	189·7
Blackberries	24	189·2
Cherries	8	200·2
Golden plums	20	191·5
Gooseberries	20	195·3
	Rolling	
Raspberries	16	194·5
Strawberries	17	188·0
Loganberries	16	200·5

The internal temperatures necessary for sterilization varied between 188 and 201° F., but as the different "cooks" used were at 4 min. intervals the actual lethal temperature might be somewhat lower than that recorded. With cherries, which heat relatively quickly, the lethal temperature was attained rapidly, but with the other berries a "stationary cook" of 20–24 min. was necessary. With the softer fruits, for which a "rolling cook" was used, the lethal temperature was obtained in 16–17 min. In most cases, the times of cooking necessary to produce these fully lethal temperatures are considerably longer than those normally used in canning practice.

Further determinations of the lethal conditions were made in factory rotary cookers. For instance, size A 1 tall cans, filled with loganberries, were inoculated with a standard suspension of spores and were collected at each of the four doors of the cooker. The 3 cans from the second door, which received a "cook" of 4 min. and showed an internal temperature of 191° F., remained infected, but the 5 cans from the third door, which received a "cook" of 5½ min. and showed an internal temperature of 199° F., were sterile.

Variation in amount of inoculum. The results of the inoculation of gooseberries, loganberries and plums with three different concentrations of spore suspension are shown in Table IX.

Table IX. *Infection in cans inoculated with different concentrations of spore suspension*

Time of "cook" (min.) ...	8	12	16	20
		Proportion of infected cans		
Gooseberries				
Light inoculation	3/5	3/5	0/4	0/4
Standard inoculation	5/5	5/5	3/4	0/4
Heavy inoculation	3/5	5/5	2/4	0/4
Loganberries				
Light inoculation	4/4	0/4	0/4	0/4
Standard inoculation	4/4	2/4	0/4	0/4
Heavy inoculation	4/4	1/4	0/4	0/4
Golden plums				
Light inoculation	5/5	1/5	0/5	0/5
Standard inoculation	5/5	4/5	0/5	0/5
Heavy inoculation	2/5*	2/5	1/5	1/5

* These cans were opened 8 months later and there were signs of slight disintegration in some berries. Thus infection may have occurred, but the mycelium died before the cans were examined.

With the light inoculum the cans were more easily sterilized than with the heavy or standard inoculum. This was in agreement with the observation that only a small proportion of the spores have a high heat

resistance; with the light suspension there would be less chance of the presence of very resistant spores in the can. The fact that there were only slight differences in the time necessary to produce sterility between the standard and heavy inocula suggested that these suspensions were sufficiently concentrated to ensure the presence of the most resistant spores. Therefore, the canning results for these inocula give a time of "cook" which is the maximum necessary to overcome any infection.

Natural infection. The method of detecting the presence of *B. fulva* spores on the fruit has been described above, and the reasons given for the view that the spores were present there in small numbers only. Assuming each infected fruit to bear only one spore, the approximate amount of natural infection entering the can may be estimated. This is included in Table X together with the data gained from canning experiments concerning the nature of the "cook" necessary to overcome this natural infection.

Table X. *Sterility in cans of naturally infected fruit produced by heat treatment*

Fruit	% infection of berries	Approx. no. of fruits in A 2 can	Estimated no. of spores in each can	Time of "cook" for sterility min.	Internal temperature in can °F.
		Stationary			
Victoria plums	26	12	3	12	178·5
Blackberries	16	80	13	20	184·7
Golden plums	10	12	1	12	181·5
Gooseberries	57	63	36	12	190·0
Black currants	29*	900	260	12	191·2
		Rolling			
Blackberries	16	80	13	8	190
Raspberries	57	160	91	6	185
Loganberries	28	80	22	8	193
Strawberries	8	40	3	10	171
,,	36	40	14	5/7 infected after 9 min.	176

* Several berries were placed in each tube in the case of black currants.

The natural infection introduced fewer spores into the can than did the suspensions used in the inoculation experiments. The highest temperature required to overcome the natural infection in all the cans used was 193° F. and a lower temperature was enough in most cases. On the other hand, when strawberries bearing considerable natural infection were given the "cook" normally used (9 min.) 5 out of 7 cans remained infected.

A few experiments were performed in factories when the fruit was sampled before filling the cans and the "central" temperature attained

in the cans during processing was recorded. Cans selected at random from the final product were examined for the sterility of their contents. For instance, gallon cans of golden plums in water attaining a "central" temperature of 185° F. (stationary cook) were sterile, although the fruit when sampled at the time of filling the cans showed 20 % of the berries infected. However, cans inoculated with the spore suspension and given the same treatment remained infected. In another case, golden plums, of which 15 % of the berries were infected, were filled into size A 2½ cans and these were removed from the doors of a rotary cooker. Five cans from the first door (cook 2¾ min.) gave a central temperature of 184° F. and all were sterile.

Inoculation into different strengths of syrup. The laboratory experiments showed that a higher sucrose concentration increased the heat resistance of ascospores. Also, various physical properties of stronger sugar solutions lead to a slower rate of heating. It was proved by experiments, the results of which are shown in Table XI, that times of cooking sufficient to sterilize in 45° Bx syrup would not do so in 55° Bx syrup.

Table XI. *Effect of density of syrup on the proportion of infected cans*

	Time of "cook" min.	45° syrup Temp. °F.	Infection	55° syrup Temp. °F.	Infection
		Stationary			
Gooseberries	20	195·3	0/4	185·0	3/3
Golden plums	20	191·5	0/5	188·2	1/5
		Rolling			
Raspberries	14	193·0	0/4	—	4/4

In such canning experiments it is not possible to separate the effect of increased heat resistance of the spores from decreased temperatures in the can and, therefore, no conclusions were reached as to the relative importance of the two factors, but the experiments showed that the combined effects were of importance in practice.

Effect of time of cooking on the texture of the fruit. As the times of cooking necessary to overcome infection with *B. fulva* were longer than those normally used, preliminary experiments on the effect of longer cooking on the final appearance of the fruit were made. This effect would be much influenced by the variety of fruit and its condition of ripeness and freshness. Such experiments were made on only one batch of each fruit, but all the fruit used was of a satisfactory state of ripeness, except the raspberries and blackberries, which were slightly overripe.

Cans were treated in the normal way and given different times of cooking, 3 cans being used each time. After being stored for 1–3 months they were opened and examined by the method used for assessing the quality of canned fruit (Hirst & Adam, 1932). In plums and soft fruits the "cook" necessary to overcome the standard inoculum softened the fruit. This softening was, however, not nearly so serious as that caused by growth of the fungus. The texture of the fruit was undamaged by cooks which were sufficient to overcome the natural infection. The harder fruits were improved by longer cooking and there was no difficulty in their sterilization.

The flavour of the soft fruits deteriorated after cooking for a time which softened the berries, but no difference in flavour was detected for different times of cooking for the hard berries. The times of cooking did not affect the colour of the fruit. Raspberries, loganberries and blackberries showed a diminishing "drained weight" of the fruit with increasing times of cooking, which suggests that the fruit had shrivelled.

(c) *Degree of control obtained*

To obtain sterility in heavily inoculated cans, which contained some of the most resistant spores, long heatings which gave an internal temperature of 190–200° F. were necessary; such heating tended to damage the texture of the fruit. With a smaller artificial infection, so that there was less chance of the presence of very resistant spores, a smaller proportion of cans remained infected after a given time of cooking. The natural infection on the berries invariably introduced many fewer spores into the container than were introduced by inoculation, and this natural infection could be overcome by a time of cooking which was not detrimental to the flavour and texture of the fruits tested. However, although only a very small proportion of the spores are highly heat resistant, there is always the chance that such a spore is present, and so long as a temperature of approximately 195° F., at which all the spores are killed, is not attained, there is the possibility of infection in an occasional can.

Spoilage in individual cans of a batch which has not received a heating which achieves this totally lethal internal temperature depends upon three factors: (*a*) the occurrence of a spore in the can which resists the heating given; (*b*) whether the spore happens to be situated in the can in a position where a high temperature is attained; (*c*) upon the packing of the fruit, which affects the temperature attained in the individual can. It is a matter of chance whether these factors coincide or not, consequently leaving the can sterile or infected.

During the 1934 season, when suggested control measures were circulated to canners, some 350 representative samples of canned fruits from factories all over the country were examined for *B. fulva*. Infection was found in 18 cans which came from only two factories. The cans contained raspberries, strawberries and loganberries—fruits for which it is most difficult to devise a satisfactory internal temperature. Samples of these fruits from other factories were sound, so that the majority of factories were obtaining good control.

(d) *Mycelial growth in cans*

Experiments showed that no growth of *B. fulva* develops in an atmosphere from which oxygen has been removed by alkaline pyrogallol; therefore the amount of mycelial growth which develops in an infected can depends upon the amount of oxygen available. The original amount of oxygen present is dependent upon such factors as size of headspace, type of fruit and temperature of sealing. Oxygen is used by the fungus, and also by chemical action (Horner, 1934), so that the amount of mycelium which develops depends on the relative rate at which oxygen is used by chemical action and fungal metabolism. The residual fungal infection in the can is also a factor determining the weight of mycelium produced. With a heavy infection the absolute increase of weight of growth is greater in a given time than with a light infection, and so the fungus absorbs oxygen which would otherwise be used in chemical reaction. This accounts for what is often observed in practice, that mycelial development, and hence the extent to which the fruit is softened, varies considerably in individual cans.

The change in composition of the headspace gas in control and infected cans of Pershore plums stored at 30° C. was determined. The headspace was 28–33 c.c. The gas compositions[1] are shown in Table XII.

Table XII. *Composition of gas in cans*

Time of storage days	Control		Infected	
	CO_2 (%)	O_2 (%)	CO_2 (%)	O_2 (%)
2	15·0	14·0	17·5	10·5
3	—	—	26·5	4·5
4	—	—	38·0	1·0
6	19·0	5·0	45·0	0·0
13	21·0	0·0	52·0	0·0
22	22·0	0·0	—	—

[1] These gas analyses, and others mentioned later, were made by Mr G. Horner with his apparatus (1934).

In infected cans the carbon dioxide concentration increased to 52 % and this was accompanied by a gradual reduction of the oxygen concentration to zero. In the control cans there was a slight increase in carbon dioxide concentration on storage and a reduction in oxygen concentration, but at a much slower rate than in the infected cans.

In all cases where series of infected and uninfected cans had received similar treatment, and had been examined at the same time, a reduction in the vacuum of the infected cans was observed. In some cases the affected cans were "blown" while the uninfected cans retained a good vacuum. This change in pressure is due to production of carbon dioxide by the fungus and indicates that a form of anaerobic respiration takes place. Some of the factors affecting the change in gas pressure and the development of mycelium are described below.

Effect of headspace. Series of cans, containing plum syrup but no fruit, were arranged so that they had different headspaces. They were inoculated with spore suspension and put through the normal process, being cooked for 5 min. at 80° C. After the cans had been incubated 9 days at 37° C. a determination was made of the pressure and composition of the headspace gas and the weight of mycelium produced. The results are shown in Table XIII.

Table XIII. *Relationship between headspace and growth of mycelium in cans*

Filling (c.c.)	560	530	500	450
Headspace (c.c. approx.)	25	55	85	135
Pressure (cm. variation from 76 cm. Hg)	− 24·8	7·1	18·1	26·1
CO_2 in headspace gas (%)	43·6	57·4	64·4	63·9
Dry weight of mycelium (g.)	0·032	0·056	0·072	0·091

The amount of mycelial growth was greater with increasing headspace. Greater mycelial growth was accompanied by more carbon dioxide production, and thus a greater gas pressure was generated in the container.

The results in Tables XII and XIII suggest that it is depletion of oxygen rather than the production of an inhibiting concentration of carbon dioxide which normally limits growth of mycelium in the can. Lack of oxygen is probably the cause of the eventual death of the mycelium.

Effect of storage temperature. Cans were filled with Richards' solution, acidified to *p*H 3 with citric acid, and it was arranged that two volumes of headspace were obtained, the one approximately 50 c.c. more than the other. The cans were inoculated with *B. fulva* ascospores and put through

the normal process, being closed at 77° C. and cooked for 10 min. at 85° C. They were then stored at different temperatures and determinations were made at intervals on 4 cans for each treatment. The data obtained are shown in Table XIV.

Table XIV. *Relationship between storage temperature and growth of mycelium in cans*

Storage temp. ^C.	Time of incubation			
	14 days	28 days	38 days	
	Dry weight of mycelium (g.)			Vacuum (in.)
	Small headspace			
15	Trace	0·008	0·004	12·1
20	0·022	0·017	0·019	9·1
25	0·021	0·020	0·021	9·2
32	0·025	0·018	0·027	7·5
38	0·035	0·018	0·032	5·3
	Large headspace			
15	0·002	0·039	0·043	9·9
20	0·052	0·033	0·031	8·6
25	0·067	0·042	0·053	6·8
32	0·052	0·037	0·042	6·7
38	0·043	0·046	0·046	5·2

It was concluded that with a large headspace maximum growth took place at 20–38° C. within 14 days, but within 28 days at 15° C. With a small headspace maximum growth was lower than with a large headspace. It was attained within 14 days between 20 and 38° C. but growth remained slight at 15° C. even after 38 days. Thus if, in practice, the cans are stacked while still warm and heat diffusion from the stack is slow, the development of mycelium will be greater than if the cans are cooled thoroughly before stacking.

V. SUMMARY

1. A survey of various fruit-producing areas in England showed that fruit and foliage were liable to be contaminated with ascospores of *Byssochlamys fulva*. Contamination was most pronounced on mummified plums and on certain fruit refuse.

2. The thermal death point of ascospores is about 96° C., but only a small proportion of spores show this maximum heat resistance. Conidia are killed by exposure to relatively low temperatures.

3. The reaction and the sucrose concentration of the heating medium, and the strain of the fungus, are factors influencing the heat resistance of ascospores.

4. Ascospores germinate slowly and irregularly, but their germination is stimulated by previous heating to about 70° C. Ascospore germination and mycelial growth take place at relatively high temperatures, in high sucrose concentrations, in acid media, and in very high concentrations of carbon dioxide.

5. Ascospores are relatively insensitive to antiseptics and to desiccation.

6. Growth is accompanied by the production of a pectin-destroying enzyme.

7. The results of laboratory experiments were applied to canning experiments and good agreement was obtained. Natural contamination on fruits could be overcome in cans by heating the contents to 195° F.

8. The amount of mycelial growth developing in an infected can depends upon the size of headspace and the storage temperature.

REFERENCES

ADAM, W. B. (1934). Diffusion of acid and sugars during processing and storage of canned fruits. *Ann. Rep. Univ. Bristol Res. Sta. Campden*, 1933–4, p. 35.

BAUMGARTNER, J. G. & WALLACE, M. D. (1934). The destruction of micro-organisms in the presence of sugars. Part I. The role of sucrose in the commercial processing of canned fruits. *J. Soc. chem. Ind.* **53**, 294 T.

BROWN, W. (1915). The action of *Botrytis cinerea. Ann. Bot., Lond.,* **29**, 313.

GWYNNE-VAUGHAN, DAME H. & WILLIAMSON, H. S. (1930). Studies in *Humaria granulata. Ann. Bot., Lond.,* **44**, 127.

HIRST, F. & ADAM, W. B. (1932). The factory inspection of canned fruits. *Univ. Bristol Res. Sta. Campden, Canner's Bull.* no. 4.

HIRST, F. & MCMASTER, N. B. (1933). Preliminary report on the heat-resistant mould *Byssochlamys fulva. Ann. Rep. Univ. Bristol Res. Sta. Campden*, 1932–3, p. 53.

HORNER, G. (1934). Gases in canned foods. Part I. Fruits. *Ann. Rep. Univ. Bristol Res. Sta. Campden*, 1933–4, p. 50.

HULL, R. (1934, 1935). Investigation of the control of spoilage of processed fruits by *Byssochlamys fulva. Ann. Rep. Univ. Bristol Res. Sta. Campden*, 1933–4, p. 64; 1934–5, p. 65.

OLLIVER, M. & RENDLE, T. (1934). Studies on *Byssochlamys fulva* and its effect on the tissues of processed fruits. *J. Soc. chem. Ind.* **53**, 166 T.

OLLIVER, M. & SMITH, G. (1933). *Byssochlamys fulva* sp. nov. *J. Bot.* **72**, 196.

SMITH, J. HENDERSON (1923). The killing of *Botrytis cinerea* by heat, with a note on the determination of temperature coefficients. *Ann. appl. Biol.* **10**, 335.

NOTE ON THE LIMITATION OF INFECTION OF WHEAT BY ASCOSPORES OF *OPHIOBOLUS GRAMINIS* SAAC. A POSSIBLE EXPLANATION

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INTRODUCTION

GARRETT (1939) demonstrated that failure of infection of wheat by ascospores of *Ophiobolus graminis* Sacc. may be due to antagonism by other organisms, and that this may be intensified by a low nutritional level for the invading hyphae. He states that "In wheat seedlings grown under pure culture conditions in sterile sand, the excretion of organic material from the growing roots may provide the accessory nutrient necessary for successful ascospore infection" and adds that "in unsterilized soil, the root excretions are readily assimilated by the general soil microflora".

In view of Garrett's experiments and the interpretation put upon them, the results of an experiment conducted in 1933 at the Imperial College of Science and Technology, London, are of interest. It was conducted in order to test whether the fungus is homothallic, as suggested by Davis (1925).

EXPERIMENTAL

In October 1933 Dr W. A. Millard of Leeds University kindly provided ascospore material of *O. graminis*. A dilute spore suspension was streaked on Petri dishes of potato dextrose agar, and a number of single spores, well isolated from any others, were transferred by means of a finely pointed dry wire needle to sloped tubes of potato dextrose agar. Forty-five spores were treated in this way and the tubes were kept in a dark cupboard. The day following removal of the spores, abundant germination of those remaining in the dishes was seen.

The tubes were observed daily for a fortnight, and by the fifteenth day colonies had not appeared. On removing the tubes a week later a minute colony was found in one tube and proved to be *O. graminis*. Each day thereafter for nearly a week one or two more tubes showed minute colonies, which afterwards grew rapidly. In all, 14 spores germinated by the end of 1 month, after which germination ceased.

Each of the 14 single spore colonies was mutiplied in a flask of a sterilized mixture of 90 g. of sandy loam soil, 10 g. of finely ground maize-meal and 50 c.c. of water. After 6 weeks, when abundant growth had occurred, the contents of each flask was used to infest a pot of sandy loam soil previously autoclaved at 15 lb. pressure for 1½ hr. Twenty-five seeds of Little Joss wheat were sown in each pot on 5 January 1934. By 26 February all the wheat plants inoculated with the various single spore cultures of *Ophibolus* were stunted and killed by the fungus, whereas control plants were healthy. Black incrustations of perithecia were found at the bases of plants in 12 of the 14 different inoculated series. In 9 of the 12 the ascospores were mature and

readily germinated. There was thus no doubt that this fungus is homothallic, as demonstrated by Davis (1925).

DISCUSSION

The behaviour of the spores when isolated was interesting. The fact that excellent germination was seen in the remaining spores on the Petri dishes following the removal of the single spores had led to the conclusion that the transferred spores must have been injured mechanically with the needle, an explanation disproved when they germinated. Apparently the only satisfactory explanation for hindrance of germination was that some substance produced by the ascospores themselves or by the perithecia, required for the germination of the spores, became so diluted on their removal to fresh agar as to result in long delay in germination.

Since ascospores of this fungus are produced abundantly under the conditions prevailing in certain countries, the question of spore germination is of practical importance in relation to dissemination of the fungus. If the results reported above can be repeated it means that ascospore infection under natural conditions is extremely unlikely, because during the period when susceptible plants are available it will be impossible to get sufficiently abundant concentration of spores for rapid germination. If a growth substance is required for spore germination, it is probably different from that which is required for hyphal growth (Padwick, 1936), since that substance is present in abundance in potato dextrose agar, on which the single spores germinated so slowly.

In his inoculations, Garrett used a concentration of 80,000 spores/c.c. This concentration is much greater than normally occurs in nature, but even so it is interesting to calculate the degree of concentration of spores. The volume of an ascospore, assuming it to be a perfect cylinder of size $100 \times 3 \cdot 5\mu$, which is somewhat larger than the figures given by Kirby (1925), is $\pi r^2 l = 3 \cdot 1416 \times (1 \cdot 75)^2 \times 100 = 962$ cu. μ. One c.c. contains $(10,000)^3$ cu. μ, so that for each of the 80,000 spores there will be

$$\frac{1}{80,000} \times (10,000)^3 \ (= 12,500,000) \text{ cu. } \mu$$

of solution, and the ratio of suspending liquid to spore volume will be

$$12,500,000 : 962 = \text{approximately } 13,000 : 1.$$

The degree of dilution of any soluble substance produced with or by the spores is thus very great.

What may probably have happened in Garrett's experiments is that the spores germinated very slowly owing to their dilution. This slow germination did not matter in the case of sterile conditions, since eventually the spores could utilize whatever growth substance was available and eventually cause infection. The delay was, however, fatal when the spores were faced with competition for nutrients by other fungi, or when the unfavourable nutritional conditions were enhanced by toxic substances produced by these or other organisms.

This interpretation may be disproved, but these results obtained 6 years ago may prove an interesting point to workers on *Ophiobolus*. In 1936 I stated "what has been demonstrated is...the importance of studying the nutritional aspect when considering questions of antagonism and growth of the fungus in soil". By a different method of approach, Garrett and I have reached the same conclusion. He says "It

is suggested, therefore, that microbiological interference with the initiation of ascospore infection is a competitive rather than an antagonistic effect, and is due to assimilation by the general soil microflora of the nutritive substances excreted from the growing and developing roots." I stated that "It is as reasonable to suppose that *O. graminis* fails to grow in unsterilized soil, at least in some cases, through lack of growth factor, or through competition for growth factor by other organisms, as it is to regard the control exercised as being entirely due to production of some inhibitory substance by these organisms." It may be that ascospores and hyphae differ mainly, physiologically speaking, in mass, or, what amounts to the same thing, "nutritional level". At any rate, we seem to have approached much nearer to the truth than we had a few years ago before it was demonstrated that *O. graminis* is a fungus of rather unusual nutritional requirements.

SUMMARY

1. Single spore cultures of *O. graminis* Sacc. produced ascospores on the host, confirming the conclusion of Davis (1925) that the fungus is homothallic.

2. Single spores removed from a Petri dish took several weeks to germinate, whereas the spores remaining in the dishes germinated within 24 hr.

3. It is suggested that a retardation of spore germination may have been due to dilution of some necessary chemical substance.

4. The significance of slow spore germination in relation to the results obtained by Garrett (1939) on infection of wheat seedlings by the ascospores of *O. graminis* is discussed.

REFERENCES

DAVIS, J. (1925). Studies on *Ophiobolus graminis* Sacc., the take-all disease of wheat. *J. agric. Res.* 31, 801–25.

GARRETT, S. D. (1939). Soil conditions and the take-all disease of wheat. IV. Factors limiting infection by ascospores of *Ophiobolus graminis*. *Ann. appl. Biol.* 26, 47–55.

KIRBY, R. S. (1925). The take-all disease of cereals and grasses caused by *Ophiobolus cariceti* (Berkeley and Broome) Saccardo. *Mem. Cornell Univ. agric. exp. Sta.* no. 88.

PADWICK, G. W. (1936). A growth factor influencing the development of *Ophiobolus graminis* Sacc. *Sci. Agric.* 16, 365–72.

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

ANNUAL SUMMER MEETING held in the University of Manchester, 8–10 June 1939. A paper reading session was convened in the Natural History Theatre, Zoology Department, on Friday, 9 June, at 10 a.m. The Chair was taken by the President, Mr C. T. GIMINGHAM.

Symposium on Applied Biology and Crop Production

The following papers were read:

I. Pedology, the study of the soil. By Prof. G. W. ROBINSON, M.A.

II. Botany and ecology in relation to crop production. By G. H. BATES, D.Sc.

III. Agricultural entomology in Yorkshire. By H. W. THOMPSON, M.Sc.

IV. Some past and present crop disease problems in the north of England. By F. T. BENNETT, Ph.D., D.Sc.

I. PEDOLOGY, THE STUDY OF THE SOIL

BY Prof. G. W. ROBINSON, M.A.

University College of North Wales, Bangor

I ASSUME that, as Applied Biologists, your interest in the soil is principally in its function as a medium for plant growth, and that you are acutely aware of the magnitude of the problems that centre in the relationships between the soil and the natural vegetation or cultivated crops that grow on it. My own special interests lie more particularly in the study of soil as a natural body, but I make no apology for this, because I believe that the problems of plant growth can be most successfully solved through a clear understanding of the nature of the soil medium, and an equally clear understanding of the mechanism of plant nutrition in soils. Whilst I recognize the immediate practical value of investigations in which the effect, measured in crop yield, of any given soil treatment is studied, it seems to me that the results of such work can be, with certain exceptions, of only limited significance and application.

During the past generation there has been a fundamental change in the methods and ideals of soil investigation. Whilst the soil was formerly regarded as a material in which crops grow, in the modern study of the soil it is regarded as a natural individual to be studied as such by the methods of pure science.

The great advance in the study of soils during recent years has been in the recognition of the importance of soil morphology. In the modern view, soil is not simply

a material but an individual with differentiation of parts. The soil individual recognized in modern soil studies is the soil profile, which consists of the succession of horizons occurring in vertical section from the surface down to the geological material from which they have been differentiated by the soil-forming processes.

Study of soil as a material, although inadequate to characterize a soil, is nevertheless necessary, if only to furnish a basis for the comparison of the horizons that make up the soil profile. It is, therefore, necessary for us to consider briefly the constitution of soil material in the light of modern knowledge.

Soil is developed from the products of weathering of the rocks of which the earth's crust is composed. In it we may recognize fragments of rock and of rock-forming minerals. The mineral portion of the soil is, in the main, the product of geological weathering and differs from it principally in being associated with the more or less altered products of decomposition of plant residues, known as *humus*. It differs also from rock débris in being the seat of a micro-flora and fauna, and in possessing a definite structure. Viewed in another way, we can distinguish in the material of soil: (*a*) unweathered rock and mineral fragments and undecomposed plant residues retaining their original structure, and (*b*) secondary products formed from the chemical weathering of silicate minerals and from the microbiological decomposition of plant residues. The secondary material formed by the chemical weathering of mineral silicates forms the *clay complex*, whilst the material formed from the microbiological decomposition of plant residues forms the humus of the soil. The clay complex is the result of processes that are to some extent geological. Indeed, in soils derived from deposits, such as Gault Clay, that have been only slightly metamorphosed, the clay complex is the product of an earlier weathering cycle. Humus, on the other hand, is the product of contemporary microbiological processes within the soil. Clay and humus occur in intimate association and together form the so-called *colloidal complex* of the soil. The colloidal complex is the reactive part of the soil, and occurs partly as independent aggregates, partly as films or coatings investing the less reactive skeletal material and causing its aggregation into compound particles or crumbs.

In the mechanical analysis of soil, the particles of which the soil is composed, after preliminary treatment to break down compound aggregates, are separated into different grades or fractions. The relative proportions of these fractions determine the texture of the soil. Broadly speaking, we may say that particles greater than about 2 mm. diameter consist of rock fragments, particles between 2 and 0·002 mm. are mainly fragments of unweathered minerals, such as quartz, felspar, micas, and ferromagnesian minerals, whilst the material less than 0·002 mm. diameter is mainly the clay complex.

The clay complex is, as we have seen, secondary material formed from the weathering of silicate minerals. It appears to consist of certain platy crystalline minerals, which may be described as hydrated aluminoferric silicates, sometimes associated with hydrated sesquioxides and, possibly, complex colloidal gels of indefinite composition.

The principal constituent of humus is a complex formed by the condensation of proteins or their hydrolytic products with altered lignins. It is relatively resistant to microbial attack, and must be considered in the main as chemically inert. Most of the unavailable nitrogen of soils must be assigned to the category of humic nitrogen.

The colloidal complex, considered as a whole, behaves as a weak insoluble acid.

In ordinary soils, this acidity is more or less neutralized by bases, the so-called *exchangeable bases,* of which the principal is calcium. In equilibrium with excess calcium carbonate a soil is *base-saturated.* On the other hand, in very acid soils, comparatively small amounts of exchangeable bases are present. In the complete absence of exchangeable bases the soil is said to be *base-desaturated,* a condition closely approached by many of our mountain soils in wet regions. The base-status of soil is reflected in the reaction or pH of the soil moisture. At complete base-desaturation the pH is approximately 4·0. Lower pH values are generally due to the presence of small amounts of sulphuric acid formed from the microbiological oxidation of organic sulphur compounds.

The prevalence of very acid reactions in the soil markedly affects microbiological decompositions. Bacterial decomposition becomes recessive to fungal decomposition and plant residues tend to accumulate at the surface as peaty raw humus, a condition that is accentuated by the more or less complete absence of earthworms. Under the same conditions, and owing to the presence of acid humus, the clay complex undergoes a decomposition whereby ferric oxide and, to a lesser degree, alumina, become mobile and are washed down from the surface horizons to be deposited at lower levels in the profile. This is indicated by a bleaching of the soil immediately below the raw humus layer and the development of rusty or yellowish colours below.

We are now in a position to consider the origin and development of soils as individuals. At this stage, it will be convenient to distinguish between rock weathering and soil formation. The distinction can be most clearly appreciated in the tropics, where the weathering of rocks has proceeded much more intensively and for a longer period than in our climate. It is not uncommon in humid tropical climates to find 50 ft. or more of weathered material overlying the rock from which it has been formed. In such cases it is plain that the soil has been developed from the weathered material by a distinct group of processes associated with, and conditioned by, the presence of a vegetative cover. The soil-forming processes are equally distinct in those cases where the parent material consists of unmetamorphosed or lightly metamorphosed sediments resulting from an earlier cycle of weathering and decomposition. In a climate such as our own, the zones of soil formation and rock weathering generally overlap. In fact, some soil profiles are so shallow that the soil profile extends down into the rock itself.

The weathering of rocks belongs more appropriately to the sphere of geology. We may note, however, two types of weathering, namely physical and chemical. In physical weathering, the changes involved are merely mechanical comminution, and are the result of such agencies as temperature changes, frost and thaw, moving water, or moving ice. In chemical weathering, which may be concomitant or subsequent to physical weathering, chemical decompositions take place, whereby certain constituents are lost and secondary products formed. We shall make no serious error in equating the clay complex of soils and clays with the secondary material formed as a result of chemical weathering.

The development of soil from weathered geological parent material is conditioned by the presence of a cover of vegetation. The dead remains of plants, consisting mainly of fibrous materials, undergo microbiological decomposition in the soil and build up a content of humus. Plant residues added to the soil from the death of roots are at once incorporated with the soil. The residues of the aerial parts of plants become

incorporated with the soil mainly through the agency of earthworms, except in the case of very acid soils, where they accumulate as a raw humus mat.

The character of the superficial accumulations of humified and semi-humified plant residues or litter, the vertical changes in humus content within the soil profile itself, and the sharpness of these changes as judged by appearance in vertical section, are of the highest importance for diagnostic purposes.

There are, however, other processes in profile development, affecting the mineral constituents of the soil. These are governed by the movements of water in the profile. In a humid climate, such as our own, there is an excess of rainfall over evaporation, and this excess is disposed of by downward percolation to the drainage. With impervious subsoils, or in the presence of high water-tables, the downward movement is limited to the zone of free percolation, and is then lateral in direction.

Material removed in solution from the upper horizons of the soil may be lost completely from the soil profile and pass into the drainage. This is the case with soluble salts of the alkalies and alkaline earths. In other cases, and particularly with material in suspension or colloidal solution, deposition may take place in a lower horizon. There is thus a differentiation into a superficial horizon, termed the *eluviated* or *A-horizon*, and a lower horizon termed the *illuvial* or *B-horizon*. The character of the eluviation differs in different classes of soil profile. The simplest case is where eluviation is purely mechanical, involving the translocation of fine material, and resulting in differentiation into a coarse-textured A-horizon and a fine-textured, often compact, B-horizon.

Where eluviation takes place in the presence of acid humus, as under coniferous or heath vegetation, the colloidal complex becomes unstable and sesquioxides, principally ferric oxide, are removed from the A and deposited in the B horizon. This is shown by a bleaching of the superficial layers and the development of orange or rust-brown colours in the B-horizon. In extreme cases, humus may be deposited at the top of the B-horizon. The fully developed profile in such circumstances is the well-known *podsol profile*. As this, or one of its variants, is the most characteristic profile under natural vegetation in northern England, Wales and Scotland, it may be useful to give a description of a typical example, such as may be found under heath vegetation or under long-established coniferous forest.

Beginning from the surface, the first horizon consists of litter in the first stages of alteration. Below this is more or less humified litter, which, however, still retains something of its original structure. These horizons together form the A_0 horizon. They are succeeded by the A_1 horizon. This varies in thickness from a mere rudiment to a few inches, and consists of the mineral soil mixed with dark structureless humus. Below it is the A_2 horizon, which has a bleached or grey appearance and, when dry, a loose or, occasionally, laminated structure. The thickness is variable, and rarely amounts to a foot. Bleached sand grains are characteristic of the A_1 and A_2 horizons. Below the A_2 horizon there succeeds, with sharp transition, the B_1 horizon, which is dark-coloured or even black owing to deposition of humus. In this horizon there is a marked increase in degree of compaction. The thickness varies from a fraction of an inch to 2 or 3 in. In some profiles, including most Welsh profiles, the B_1 horizon is absent or indistinguishable. Below the B_1 horizon is the B_2 horizon, characterized by yellow, orange, or rust-brown colours owing to deposition of hydrated iron oxides. The B_2 horizon varies in thickness from a few inches to 2 ft. or more, and merges

with varying distinctness into the *C-horizon*, which is the parent geological material.

Most of the well-drained soils of the humid north and west of Britain are variants of the podsol profile. A profile such as the one described may be termed a developed or mature profile. Where the natural profile has been changed, owing to cultivation, we have agricultural profiles, in which may be distinguished grassland and arable profiles, respectively. It is obvious that when a virgin soil is cultivated, all horizons down to the depth of tillage are mixed together and lose their identity.

Following on cultivation, erosion may remove some of the surface horizons, thus giving truncated profiles. Many of our Welsh upland soils are of this class, the present top soil representing the former B-horizon. Apart from modifications induced by cultivation and erosion, there are variations in the degree of maturity. Profiles in the earlier stages of development may be termed *immature*. Even given the same conditions of development, there are also variations depending on the character of the parent geological material. Podsolization is favoured by a low mineral status in the parent material. Consequently, development will be more pronounced on highly siliceous sands with low base content than on materials such as shales, which, although not calcareous, may contain reserves of weatherable minerals. Where parent materials are calcareous, podsolization cannot occur so long as calcium carbonate is present in the soil horizons.

The problem of soil classification has occupied considerable attention for many years, and it cannot yet be regarded as satisfactorily solved. Perhaps the principal difficulty has been the incompleteness of our knowledge of the soils of the world. A system of classification that is adequate for the soils of a region or country is generally found to be inadequate when applied to other regions. Since it is only during comparatively recent years that methods of characterizing soils as individuals have been developed, the materials for a system of classification are still incomplete. Nevertheless, certain world groups have been recognized and the broad lines of a classification may be given.

We may distinguish in the first instance three great classes or groups. First, there are *soils with completely leached profiles*. These are soils with free drainage in humid climates. Secondly, there are *soils with incomplete leaching* owing to the predominance of evaporation over rainfall. There are the soils of arid climates. Thirdly, there are *soils with impeded or restricted leaching*, occurring both in humid and arid climates.

The first group may be subdivided according to the presence or absence of an acid humic surface layer. In the presence of such a layer, we have the *podsols* and their congeners. Where an acid humic layer is absent, we have the *brown earths* or *brown forest soils* of temperate climates and the *red loams* and *lateritic soils* of the humid tropics. Such soils, although acid and free of carbonates, do not show bleached A-horizons and sesquioxide accumulation in the B-horizons.

The second great group may be subdivided according to differing degrees of leaching, varying from the *tshernosems* or *black earths* to the *desert soils*, the presence of an horizon of calcium carbonate accumulation being a constant feature.

The third great group can be at once subdivided according to the presence or absence of sodium salts. In the former case we have the saline and alkaline soils; in the second case we have a range of soils varying from so-called *gley soils*, characterized

by grey colours and rusty mottlings or patches in the region of the water-table, to the *peats*.

The categories distinguished by this primary classification may be further subdivided according to the stage of development of the soil profile, recognizing such variants as *eroded* and *agricultural profiles*. Finally, distinctions may be drawn on the basis of parent material and texture.

It will be realized that the number of possible varieties of soil is very large. The problem of classification is rendered still more complicated by the occurrence of transition types. The final categories have not the definiteness of genera and species in plants and animals, but often represent ideal stages in a continuous succession, analogous to the ideal granites, syenites, and gabbros of the petrologist.

I feel that I should be avoiding an important aspect of pedology if I omitted some discussion of the mutual relationships of soil and vegetation. The problem presents itself to you as applied biologists in such questions as these. What information about the soil should be collected in order to specify soil conditions in ecological studies? What characters of the soil are critical for plant growth? What soil characters determine the occurrence and distribution of ecological units?

Before attempting to arrange the different soil characters in order of importance, I will briefly enumerate the headings under which information is desirable in describing soils from the point of view of their effect on plant growth.

In the first place, climatic and topographical data must be recorded, with special attention to micro-relief. With these data may be included information on the hydrological conditions and a general statement of the superficial geology. I assume that biologists will collect all data on vegetation.

The next step is to describe the actual soil profile. This can only be examined in a section, best obtained by digging out a hole or trench. The depth necessary to include the complete profile varies, but would rarely exceed about 4 ft., to include all the pedological horizons. It is generally advisable to secure a natural face to the vertical section by breaking off the surface. Examination of a face smoothed or compressed by digging implements may give misleading results. Inspection will now enable the observer to recognize the different horizons down to the parent material. These horizons should now be described in succession from the top. For each horizon, data should be recorded concerning thickness, colour, texture, stoniness, organic matter, structure, and moisture conditions. If possible, the reaction should be determined on the pH scale, and the occurrence of carbonates noted, if present. Any secondary depositions such as rust marks in root channels, and concretions or deposits of iron oxides or manganese dioxide should be recorded.

Certain laboratory data are also desirable, and samples for analysis should be taken from each horizon. It should not generally be necessary to carry out complete analyses, but it would be desirable to determine the organic carbon, the pH, and, if possible, the mechanical analysis. The organic carbon multiplied by the conventional factor 1·724 gives a measure of the organic matter. Loss on ignition as a measure of organic matter is entirely untrustworthy.

In conclusion, I will attempt to indicate the soil characters which appear to me of most importance for plant growth and which would appear to determine the occurrence and distribution of different vegetational units, so far as these are determined by soil conditions.

So far as the growth of natural vegetation is concerned, it would appear that two soil factors are of outstanding importance, namely, (1) the air-water regime within the soil, and (2) the base-status.

I group aeration and moisture together because excess of the one implies defect of the other. In the last analysis, practically all limitations imposed by the soil on the growth of plants operate through the air-moisture regime. Thus, shallowness implies liability to drought through restriction of root development. Coarse texture implies low moisture-holding capacity and excessive oxidation of organic matter. Heavy texture implies deficient aeration and restricted root development during the wetter months and liability to drought during the summer.

The importance of the air-moisture regime is evident when agricultural soils are considered. Human interference can effect much by modifying unfavourable soil conditions and building up fertility. It is safe to say, however, that our most fertile agricultural soils are those in which the factors water-supply and air-supply operate most favourably. Such soils are found by experience to repay good cultivation and manuring and therefore acquire good plant food status.

The other factor that appears to be of critical significance is the base-status of the soil. It is possible to view this property of the soil from more than one standpoint. Most usually the base-status of the soils is visualized in terms of the reaction of the soil moisture as expressed by the pH value. It is true that the pH does reflect the acid-base regime of the soil. It is equally true that excessively acid and excessively alkaline reactions may directly exert an adverse effect on plant growth, and that the critical reaction may vary for different plants. One must not, however, accept the significance of pH too uncritically. For one reason, the accurate determination of the pH of the soil is a matter of considerable difficulty. Apart from this, however, it must be noted that the pH of the soil is an expression of a property which is governed by a number of factors, principally by the nature and amount of the colloidal material present, and by the nature and amount of the associated exchangeable bases. It may be, therefore, that the critical factor for plant growth is not actually the pH, but the base-supply. From my own experience of agricultural soils in North Wales, I am disposed to attach greater importance to the actual amount of lime in the soil than to the reaction as measured by pH. And so, whilst pH data may give useful indications, they should be used with great caution in ecological studies.

Equal caution is needed in the use of oxidation-reduction potential measurements. Whatever may be its significance, the determination of oxidation-reduction potential is beset with enormous difficulties. In fact, some workers have even come to the conclusion that oxidation-reduction potentials only represent inaccurate pH determinations.

In the present state of our knowledge of the soil, we are still far from being able to effect a complete correlation between soil profile characters and plant growth. This is due as much to imperfections in our knowledge of plant nutrition as to the inadequacy of our pedological knowledge. The principal problem of the pedologist is to understand, describe, and classify his material. In this way he can most efficiently contribute to the solution of the problems of the growth and distribution of plants.

(*Synopsis*)

II. BOTANY AND ECOLOGY IN RELATION TO CROP PRODUCTION

By G. H. BATES, D.Sc.

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IN reviewing so wide a field it is not possible to deal with every aspect of the subject in detail. Probably the most satisfactory procedure is to consider those contributions which, in the writer's opinion, are the most important from the cultivators' point of view.

In plant breeding the application of Mendelism has been a great advance on the methods of the past, it is more economical and more productive of results. On the other hand we appear to have gone as far as Mendelian methods can take us, and have arrived at a point where we have rung the changes on all the available material.

It is probably for the above reasons that such a wave of optimism has swept through the ranks of geneticists after the advent of the colchicine method for the production of polyploidy. It is possible to produce fresh material to work upon, but whether results up to date can be regarded as being more than encouraging is a matter of doubt.

There has been a revival of the study of graft hybrids and attempts have been made to produce disease immune potatoes by building up periclinal chimaeras with an "outer skin" of a disease resistant variety. We have also learned to understand the constitution of some of our existing varieties.

Greater attention is being given to root studies. For obvious reasons our knowledge of underground organs lags far behind that of aerial parts. New technique is allowing for observations on the development and reactions of roots. This has been made possible by the introduction of glass chambers and shafts. It is possible that the plant breeder may be able to make use of root characters in producing strains resistant to drought, disease and lodging, once he has been enabled to identify those characters.

Work on growth promoting substances has cast light on growth mechanism flowering and tuber formation. Results as a whole are conflicting and contradictory, as may be expected in a new field. Some concrete achievements have emerged and synthetic products are available for the stimulation of root development.

Investigations on dormancy and methods for breaking the resting period have not received the prominence they deserve. Apart from the well-known treatment of bulbs, there appear to be distinct possibilities for the application of chemical treatments for breaking dormancy to potatoes.

The new technique for plant injections has opened a wide field of investigation. There is a better understanding of the vascular system and movement of water, owing to the fact that certain dyes may be taken into the plant without causing injury. It is hoped that the system may be used for diagnostic and curative purposes on a greater scale than at present. The results of investigations carried out in this field cast valuable light on problems which have arisen in connexion with a new method of bracken destruction by the application of sodium chlorate to the cut surfaces of the stems.

In the sphere of ecology there is need for a great enlargement of the work done on

the aut-ecology of certain weed species. A good deal of evidence has been produced pointing to the relationship between weed flora and cultivations.

In grassland improvement it might be said that the whole of the work carried out at Aberystwyth is really applied ecology. The production of indigenous strains and types suited to the particular habitat has been a great step forward. Methods of improvement of hill land show an appreciation of the concept of sequence and climax.

(Synopsis)

III. AGRICULTURAL ENTOMOLOGY IN YORKSHIRE

By H. W. THOMPSON, M.Sc.

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ENTOMOLOGICAL problems of one Advisory Province must, generally speaking, bear a close resemblance to those of other corresponding areas. Consequently, a brief outline of the type of advisory work met with in Yorkshire will, it is hoped, give an indication of the general scope of agricultural entomology and its relation to crop production.

Yorkshire is large and varied, practically every geological formation occurring in the county, with the result that practically every type of soil exists and, therefore, every type of farming. Physically the county is made up of four upland masses with lowland areas between and about them. To the west of the county lie the Pennines, almost continuous from north to south but broken by the Aire Gap which allows the passage of river, canal, roads and railway. In the east of the county we have the North Yorkshire Moors which are largely heather covered, and farther south, in the East Riding the Wolds, which 100 years ago were mainly rough sheep grazing land but are now 75% arable and form an important cereal-growing area.

All the central part of the county, and roughly half of it, is taken up by the Plain of York, which extends from north to south of the county. Running eastwards from the plain there are in the north, the Vale of Cleveland, in the centre the Vale of Pickering and in the south on Humber side the Holderness area which includes the land adjoining the tidal rivers, Ouse and Derwent, where extensive warping has been carried out, mainly 30–60 years ago, but some of it in recent times.

MAIN CROPS AND PESTS OF THE AREAS INDICATED

(1) *North Pennine region*

This area which is largely mountain limestone, with some millstone grit, is an important grazing area, and in the main has no big entomological problem. Pests which occur at intervals are leatherjackets and garden chafers causing turf injury, antler moth and on one occasion fescue aphis causing severe herbage injury, and the heather beetle on grouse moors, which causes widespread injury.

(2) *Southern Pennine region*

This area includes the industrial south-west region of the county and lies on millstone grit and coal measures' formations. It has large areas of allotments and extensive market gardens on which all the common vegetable pests occur and are

troublesome at times. Important special crops which are grown intensively locally are broccoli and rhubarb. On the former, cabbage-root fly attacks are often serious, although now checked to some extent by more general use of deterrents. Rhubarb has few pests, but one, the stem eelworm (*Anguillulina dipsaci*), which has now been shown to cause a crown-rotting condition, is a serious local problem. As this eelworm has a very big host list, the attack on rhubarb introduces complications in the selection of a rotation on land on which rhubarb is to be grown for forcing.

Peas for picking green are largely grown on the fringe of the industrial area, and on this crop heavy attacks of the pea thrips frequently occur. Early peas are often followed by wheat, and it is found that attacks of wheat bulb fly are liable to follow the partial fallowing after the early ploughing of the peas. Such attacks are liable to be severe, as much of the land in this area is too light to be ideal for wheat.

North Yorkshire moors.

In this part of the county conditions and pests are similar to the north Pennine region and there is little as a rule of entomological interest. At one centre, however, watercress is grown on a large scale, and mustard beetle attacks have proved a very serious problem in the past. *Derris* could not be used owing to adjoining fish hatcheries, but satisfactory control has been obtained by pyrethrum spraying.

East Riding wolds.

This interesting region is an important barley-growing area, and on this crop gout fly attacks occur from time to time, but usually appear to be more severe on the lower ground around the wolds rather than on the top.

It is common practice here to plough out clover in late autumn and sow wheat at once, and severe frit fly attacks on winter wheat often result from the migration of the larvae from the grasses in the leys to the wheat. Summer ploughing of leys which would prevent such an attack is not practicable for various reasons on the wolds.

In 1938 attack of the wheat shoot beetle (*Helophorus nubilus*) were severe on crops which owing to an unfavourable seed time and winter were very backward. This pest was recorded as early as 1920, but is not usually severe. Attacks follow the ploughing out of clover, which is commonly sown alone for sheep grazing.

Swedes complete the cropping of the wolds. Owing to the low rainfall of this area and its soil characteristics, early growth of this crop is often very slow and in consequence attacks of turnip flea beetle are frequent and severe. Seed dressings to prevent attack are often used, not always successfully. The open chalky soil favours earwigs, which are very prevalent and often cause injury to the swede crop.

The Plain of York

This region is, generally speaking, very fertile and here farming reaches a high standard. All farm crops are grown, the most serious pest being the frit fly. Winter oats are not grown to any great extent. In some years, when weather conditions make it necessary to sow spring oats late, or when the young plants are checked in growth, the danger of severe attack is great.

In the northern part of the plain, oats are grown on a very large scale, often at short intervals and stem eelworm attacks frequently occur. During the last few years the cereal strain of the root eelworm (*H. schachtii*) has been found to be prevalent also.

Sugar beet is grown to the extent of about 30,000 acres in the centre of the county. In the last few years the serious beet pests have been cutworms, mangold fly, and black aphis. No case of "beet sickness" has been met with so far in the county and, with present restrictions on the too frequent growing of beet, should never develop into a serious problem in the county.

Large acreages of carrots are grown south and east of York, and carrot fly causes heavy loss in some years. Deterrents are now being tried on a field scale by some growers, but the general opinion appears to be that over a period of years losses would not be sufficiently heavy to justify annual applications of insecticides.

The southern part is the main potato-growing area, and extends to the warp areas around the Ouse and Derwent. This latter area is very suitable for wheat as well as potatoes, and on many farms these two crops were grown in alternate years in the past with the result that much of the land developed "potato sickness". The need for longer rotations is, however, generally recognized now, so that potato crop failures due to eelworm have become rare on a farm scale.

Many farmers in this area submit soil samples each year for cyst examination before arranging their potato acreage. The remarkable power of recovery of wheat grown in this district overcomes to a large extent danger of loss from wheat bulb fly, which is prevalent.

Large acreages of field beans are also grown on the Humber side and usually do extremely well, although in some years stem eelworm (*A. dipsaci*) causes heavy loss to this crop.

Fruit

Good fruit can be grown in the county; there are some 3000 acres of orchards, but very few fruit growers on a large scale. Generally speaking fruit culture is rather backward, few growers have a regular spraying programme, and many do little or no spraying. Consequently heavy aphis attacks are common. Other common pests are winter moth, capsid bug in the southern half of the county, codling moth and apple sawfly, the former having become more prevalent during the last few years. Apple blossom weevil has been found at two centres only, and must have been absent or nearly so until the last year or two.

Attacks of magpie moth and gooseberry sawfly on bush fruit are of general occurrence.

Strawberries are grown commercially on a large scale near Hull, and on this crop aphides, red spider, and strawberry eelworm are the main pests.

Glasshouse crops

Glasshouse cultivation has developed enormously in the last few years, particularly in the amount of Dutch houses with removable lights. Tomatoes, cucumbers and chrysanthemums are grown extensively, and all the common pests of these crops are present. In addition we have one special problem in Yorkshire which is the attack of the root eelworm (*H. schachtii*) on tomatoes causing "tomato sickness". This attack was first noticed in 1928, and we now have records of attack at almost 200 centres. An account of this attack has been published (Johnson & Thompson, 1936) and need not be re-stated; since it was written further observations have been made on the effect of change of soil, of steam sterilization on the cyst content of the soil, and on the effect of chemical dressings applied to infested soil.

Control

(1) A good crop can be obtained by changing the soil to a depth of 15 in. This may allow two tomato crops provided the subsoil infestation is not heavy, and is useful in small houses and those awkward to steam.

(2) Steam sterilization when done thoroughly is effective and should allow two satisfactory crops to be taken, but not more, and some growers favour a rather lighter steaming each year.

(3) Dutch houses may be moved to eelworm-free sites; this can be done at a less cost than steam sterilization and in the absence of eelworm such houses are usually kept on one site for 10 years.

(4) In houses where steaming is not possible, nor regular change of soil, some safeguard can be obtained by watering the beds with corrosive sublimate solution at 1 in 250 strength, using 1 gal./sq. yd. 3 weeks before planting.

Attacked plants can be helped to some extent by paying particular care to cultural details or by layering into or mulching with eelworm-free soil to promote surface rooting.

In Yorkshire the areas where "tomato sickness" occur coincide with the main potato growing part of the county, where "potato sickness" is also prevalent. Cases have come to notice where both Dutch and permanent houses have been erected on "potato sick" land, and "tomato sickness" has developed on the first year's crop. The mature tomato strain cysts appear indistinguishable from the potato strain cysts; they fall within the same size range and are believed to be identical.

For general purposes in giving advice, the presence of 10 viable cysts per 20 g. of dried soil is regarded as the danger mark, and this figure has worked out well in practice.

With an infestation below that figure a satisfactory crop may be obtained; if far above it, severe sickness is likely to occur.

REFERENCE

JOHNSON, L. R. & THOMPSON, H. W. (1936). Tomato sickness in Yorkshire. *J. Min. Agric.* **43**, 48.

IV. SOME PAST AND PRESENT CROP DISEASE PROBLEMS IN THE NORTH OF ENGLAND

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THE mycological problems in the four northern counties do not differ in kind from those in other provinces, but they are fewer in number and of comparatively greater local economic importance. This arises from the fact that the kinds of farm crops and horticultural activities are restricted by geographical and climatic influences. The geographical features are the hill masses of the Cheviots and Northern Pennines, somewhat T-shaped, with the Cumbrian mountains occupying the greater part of the space in the left arm. In these uplands sheep farming is practised, there is little arable

land, rarely as much as 5% of the farm being tilled, and the small arable crops are consumed at home. In the more extensive arable area, the eastern slopes of the Pennines to the North Sea, climatic conditions have a restrictive influence. The mean average temperature is low; at the Northumberland County Farm (Cockle Park), typical for the area, the mean maximum is 52·5° F., and the mean minimum 39·5° F. Night frosts are common in May, and at times occur to the end of June. In spring and early summer there are "sea frets"—low cloud conditions, damp and sunless, which retard the drying and warming of the soil. Under these conditions a large proportion of land is under permanent pasture or long leys which provide no mycological problems; the arable crops are of comparatively few kinds, horticulture is limited, and fruit growing on a commercial scale is absent. As the kinds of crops are relatively fewer the mycological problems concerning them assume comparatively greater importance, and call for special attention in relation to the local climatic conditions and farm practices. Of the farm crops the problems are concerned with swedes, cereals and, to some extent, potatoes, and the following notes indicate past, present and probable future developments.

Club root (Plasmodiophora Brassicae) was formerly the limiting factor in root husbandry. Control of this disease by lime, first demonstrated at Cockle Park, is now the standard practice everywhere. Frequently in the north, however, it is impossible to apply economically the amount of lime required for good control of club root on these notoriously sour soils. The problem was investigated, therefore, as to the use of resistant varieties, and the use of soil dressings which would assist this insufficient liming. As regards the latter, no substance has yet proved successful and economical under field conditions. As regards the former, resistant varieties were tested for several years, and finally nine most promising ones were tested extensively under two sets of conditions: (1) in West Cumberland on badly contaminated marshy land in a wet season; under these severe conditions the resistance of all varieties broke down: (2) under less severe conditions in Northumberland, where Bangholm and Wilhelmsburgher proved markedly resistant. These less severe conditions are those generally encountered, and the control measures applicable to the area are as follows.

When the full lime requirement of the soil cannot be met, liming is done just before the root crop to improve the tilth and surface drainage of the heavy clay soils, thereby reducing incidence and severity of disease, the use of basic slag for phosphates, and the growing of a resistant variety.

Dry rot (Phoma Lingam) occurs widely and severely; on some farms with short rotations the growing of swedes has been abandoned on account of this disease. On the farm the trouble arises from a combination of affected seed and contaminated dung. Preliminary observations only have been made as yet, in preparation for attempting control on a farm next year by seed treatment and the growing of roots without dung for several years, this necessitating a radical change in general farm practice. One observation showing the possibility of loss from infected seed may be quoted. Seed from two stocks of one variety from the same merchant were grown alongside each other on a field which was not contaminated in any way; one stock of seed gave 60% of diseased roots, the other stock 1%.

Brown heart of swedes. This trouble was first observed in Cumberland in 1931, and was associated with heavy liming of fields near to a free supply of waste lime. Our first field experiments with dung and lime were failures, the reasons not being known until

after the Canadian discovery in 1934 that brown heart was a result of boron deficiency in the soil. Thereafter other trials were made in this area, culminating in 13 field plots on various types of soil in districts from North Cumberland to South Westmorland. An account of this disease and the trials was published in the *Journal of the Ministry of Agriculture* (1939), **45**, 1232. So far as concerns this Northern Province the principal points emerging were as follows. Boron deficiency is not known in Northumberland and Durham; it occurs in the lighter soils of the west. Boron deficient soils do not occur uniformly throughout any district or over any farm, so that routine treatment cannot be recommended. The trouble is eliminated by use of borax or boronated basic slag, at a cost of from 2*s*. 6*d*. to 3*s*. 6*d*. per acre, and by boronite (a dressing ready for application from a manure distributor) at 11*s*. per acre. Part of this problem still remains, namely, the observations of other crops on the treated areas and of the need for treatment in the following swede crop.

Cereal crop problems

At least three-quarters of the cereals in this Province is oats, and since the dressing of seed with mercurial dusts is now widely practised the usual diseases of oats do not cause serious loss. A problem which does arise frequently is a form of grey leaf on land suffering from colliery damage; trials of manganese sulphate dressings often fail to cure the trouble, and it is assumed that other conditions in these defective soils induce this physiological defect in oats.

Barley and wheat are grown to considerable extent in the most suitable districts of Northumberland and Durham, but the fall of arable area from 80 to 30% has correspondingly reduced cereal husbandry. The usual diseases are always with us, but yellow rust and *Fusarium* disease call for special mention. Yellow rust is here favoured by the high condition of land after the long days, and by the use of susceptible varieties such as Standard Red, Squarehead's Master, Wilhelmina, etc. Little Joss is unsuitable for the district, and is grown in one small area only. *Fusarium* disease is not markedly dependent on previous cropping, and is favoured by the sour soils, cold and wet spring weather, and by frequent wet harvests. It attracts most attention in spring when crops fail to respond to favourable growing conditions. The investigations have been concerned hitherto with the species of *Fusarium* and their pathogenicity toward cereals; the outstanding discoveries were the prevalence of two pathogens, *F. culmorum* and *F. avenaceum*, and the occurrence in this country of *Gibberella Saubinetii* and *F. nivale*. The present and future investigations concern the disease under natural conditions in field plots, but of these nothing can yet be said with certainty. *Ophiobolus graminis*, the other foot rot organism, occurs regularly, but to small extent even where two or three cereal crops are taken in succession after long leys. Garrett's recent researches indicate that green manuring well in advance of corn sowing is one of the best means of controlling *Ophiobolus*, and we can now see a reasonable explanation of the relative freedom from this disease in the north, namely a combination of more or less acid soil, successively lower susceptibility of the kinds of cereals grown in succession, and the green manuring by long leys.

Potato crop problems. The chief potato-growing districts lie just around the industrial districts of the Tyne, Tees, and West Cumberland. New seed gives satisfactory results for 3, 4 or 5 years, consequently growers are content to renew stocks from Scotland as required. Spraying against blight (*Phytophthora infestans*) is practised

to a small extent only, the growers contending that it is more economical to risk an occasional bad season than to spray every year.

The problem we undertook in relation to potato husbandry in the north was the production of seed potatoes in the Eden valley where soil and climate are specially suitable. A growers' association now functions automatically, and seed potatoes produced here compare most favourably every year with the best of seed from elsewhere. A development of this work is the improvement of the existing stocks of the "crisp making" variety Bintje; after several years' selection two fixed types have been segregated and are now being grown on a large scale for factory trials. The improvement in these nucleus stocks maintained in Cumberland becomes an advantage also to those who multiply them and grow them on a large scale elsewhere.

Market-garden crops

For reasons previously mentioned market gardening is confined to the districts surrounding industrial centres, and comparatively few types of crops are grown. To avoid misconception it should be mentioned that during the past year advice was given concerning more than fifty different kinds of horticultural plants. Outstanding crops are brassicas, leeks and tomatoes, and these provide some special problems of particular importance in this area. First came the insistent demand for information about leek blight. The leek growers are mainly small gardeners who specialize in exhibition leeks, some of whom have improved and maintained their own stocks for 50 years. Just prior to publication of the investigation of the disease and control measures, Foister, working independently, also ascertained the causal organism and named it *Phytophthora Porri*. Perfect control of the disease is obtained by spraying the plants with Bordeaux mixture, in spite of the fact that the organism persists in the soil and a soil treatment might appear more appropriate. The reasons are given in publications on this subject.

Amongst the brassicas a problem under present investigation is a chlorotic condition resembling a mosaic, but not, according to Dr Kenneth Smith, of virus nature. It occurs throughout a district, causing the dying off of winter and spring greens under frost, and stunted useless heads in cauliflower and broccoli. The investigation is based on soil dressings and exchanged transplants. Awaiting attention is a problem concerning cauliflowers and broccoli of pink colour and bitter taste.

Amongst tomatoes all the usual troubles are encountered, but the outstanding one is root rot associated with *Colletotrichum*. Its prevalence and severity may be linked with the fact that soil temperatures remain low until comparatively late in spring, and growers mistakenly follow the practices of warmer districts. Advice given from Cheshunt is followed, but not always with success, so it is hoped to investigate this problem under local conditions at the Cumberland Horticultural Station in the near future.

The list of problems in the Northern Province would not be complete without mention of the diseases of fine turf as cultivated for golf course and bowling greens. The best of such greens are laid with sea-marsh turf, and the first and best of such turf came from Cumberland. It is appropriate, therefore, that the diseases should be investigated here. The problem in the first place was to ascertain the reasons for the failure of fine turf greens, and this led to the recognition of diseases. Of these there are three common ones, viz. *Fusarium* patch, *Corticum* rust, and dollarspot. For the first

two diseases control measures have been devised and are practised successfully; for the last named satisfactory control has not been obtained—the British organism does not respond to the treatment utilized in the U.S.A. There are instances also of *Rhizoctonia* disease, the American "brown-patch" in Britain, and isolation and comparison of strains are nearing completion. The problem in hand in connexion with these diseases is to devise one treatment for all to obviate need of diagnosis and differential treatments. To this end some 20 chemicals and proprietary articles have been examined as to their toxicity toward, or capacity for, inhibiting the growth of the various fungi and their different strains. This laboratory work is nearly completed and will later be carried into practical trials.

It may be gathered from the foregoing outline that, although many agricultural and horticultural crop plants are scarcely or not at all grown in the north, there is ample scope for investigation of local problems and for bringing mycological science into practical application.

REVIEWS

Diseases of Fruits and Hops. By H. Wormald. Pp. 290. London: Crosby Lockwood and Son, Ltd. 1939. 17s. 6d.

For almost the whole period of his scientific life Dr Wormald has been engaged in research on diseases of fruit trees and hops, and, since 1923, he has been in charge of pathological work at the East Malling Research Station. His experience of these problems must be almost if not quite unique and there can be but few growers' troubles of which he does not possess first-hand knowledge. His own numerous publications have contributed notably to our understanding of these problems and he has now increased our debt by the production of this valuable work. This book, together with Massee's *The Pests of Fruits and Hops* (see *Ann. appl. Biol.* 1937, **24**, 667) forms a splendid survey of the fruit-growers' troubles. The book is written essentially for the grower and aims at describing symptoms rather than pathogenic organisms. Its arrangement facilitates identification of the diseases which are grouped according to the host plants, and then under the various organs on which the symptoms are to be seen.

Ch. I is introductory dealing with factors conducive to health or disease in plants, and Ch. II is a brief but clear and practical account of fungicides and their application. Some diseases such as apple scab or apple canker are restricted to particular hosts, but others such as crown gall or grey mould affect a number of different plants. These more general diseases are dealt with in Ch. III although they are mentioned again under hosts, with special reference to particular symptoms or the effect on those plants. The following ten chapters are then devoted to the more special diseases of the large and small fruits, two chapters being given to the apple, one to pear, quince and medlar, two to the stone fruits, three to bush fruits, one to the strawberry, and one to the grape vine, fig, mulberry, walnut and cob nut. Hops are not in the same category as the fruits mentioned above, but Dr Wormald was wise in his inclusion of Ch. XIV which is an excellent account of diseases of this plant. A final and valuable chapter is devoted to fruit tree diseases such as fire blight, internal cork, etc., which are important in other countries but not yet recorded here.

Appended to the discussions of many diseases are selected references, and the book concludes with indexes to popular names and control measures, to scientific names, and to authors. In addition to twenty-four text-figures, one or two of which are not quite up to standard and in a few of which no magnification is stated, the work is illustrated by forty plates each containing from four to six photographs. The latter, taken by Dr Wormald himself, or by Miss Cornford under his supervision, are one of the finest series of illustrations yet published in any volume on plant diseases. The work is written in a simple, clear style and there can be no excuse for any grower failing to recognize particular diseases described; control measures advised are practical and feasible. In less skilful hands a book planned as is this would inevitably have contained much repetition but Dr Wormald has largely been successful in avoiding this.

It is difficult to find points of serious criticism in the work. A tabular key based on symptoms might have been a useful addition, but the author's arrangement and descriptions are so clear that it is really unnecessary. Other points noted may be mentioned. The author is a little inconsistent in giving spore measurements sometimes as averages, sometimes as maximum and minimum. In his citation of references he does not adopt the standard abbreviations as given in the *World List of Scientific Periodicals* and his own abbreviations are not consistent. Although the book is not over-burdened with references some of those cited seem unnecessary, whereas others that one might expect are omitted. On many of the plates there is a considerable amount of wasted space.

Reviews

The book will be useful not only to growers, but also to students as a complementary volume to standard textbooks of plant diseases. It contains a foreword by Dr Pethybridge and, very appropriately, is dedicated to Prof. E. S. Salmon.

WILLIAM B. BRIERLEY.

Nytteplanter. By K. GRAM, H. J. JENSEN and A. MENTZ. Pp. v + 503. Copenhagen: Messrs Gyldendal, Ltd. 1938. 9.75 kr.

During the last two decades valuable works dealing generally with the field of economic plants have been written by American, British, French, and German authors but this is the first Danish treatise to be published.

The contents of the book are arranged in five main divisions: I, Food plants (pp. 233; starch; oil-nuts; sugar; roots, vegetables and salads; fruits; forage): II, Aromatic plants and stimulants (pp. 72; including beverage plants): III, Medicinal, drug and poisonous plants (pp. 50): IV, Technical plants and products (pp. 118; fibre; cork and timber; charcoal; rubber; oil; wax; saponin, soda, iodine, etc.; gum; scent; tannin; dye): V, Plants used in conserving or improving soil (pp. 12).

The plants, their culture and economic uses, receive brief but adequate description, and the text is illustrated by 300 excellent photographs and line-drawings. There is a bibliography of 72 citations and an index.

WILLIAM B. BRIERLEY.

Poisonous Plants of the United States. By W. C. MUENSCHER. Pp. xvii + 266. New York: The Macmillan Co. 1939. 15s. 0d.

Part I of this book deals with the nature and classification of poisonous plants and occupies only 18 pages. It is merely a very brief summary of the generalities of the subject—the chemical nature, properties, and physiological action of the toxic principle, and the conditions under which poisoning is produced, and then brief accounts with lists of plants causing dermatitis, or photosensitization, cyanogenetic plants, introduced poisonous ornamental plants, plants producing poisonous seeds, seleniferous plants, plants producing undesirable flavours in milk and milk products, and plants causing mechanical injury. The author must have gone to considerable trouble to review and summarize the data of this portion of the subject, and it is a great pity that he did not amplify his consideration of the problems, since an up-to-date and comprehensive survey of these more fundamental issues would have been extremely valuable.

Part II (pp. 19–239) is an accurate and concise account of poisonous plants arranged by family according to Engler's system, about 400 species representing 68 families being included. Fungi are omitted but all the vascular plants of the U.S.A. that are known to cause poisoning when eaten, by contact, or by mechanical injury, to man or animals, are included. In general each plant is treated as to names, description, distribution and habitat, poisonous principle, conditions of poisoning, symptoms; and in a few cases simple remedies are indicated or references to treatment are cited. The consideration of each species is brief and to the point and, as in the author's previous book *Weeds* (see *Ann. appl. Biol.* 1936, 23, 662), the text is illustrated by numerous original and beautiful full-page line drawings by Mrs Helen Hill Craig. There is a bibliography of 231 references and an index.

The book is naturally in very large part based upon the literature of the subject, but it is an extremely good compendium, and it will serve as a useful companion volume to the recent second edition of Long's *Poisonous Plants on the Farm.*

WILLIAM B. BRIERLEY.

Introduction to the Botany of Field Crops. By Prof. J. M. HECTOR. (South African Agricultural Series, Vol. 16.) Vol. I: Cereals. Pp. xii + 478 + xiii–xxxiv. Vol. II: Non-Cereals. Pp. viii + 479–1128 + ix–xxxiii. Johannesburg: Central News Agency, Ltd.; London Agents: Messrs Gordon and Gotch, Ltd. £3. 10s. 0d. net per set.

In these two volumes Prof. Hector deals with those families that contain plants cultivated as field crops in temperate, tropical, and sub-tropical countries.

Vol. I is devoted to the cereals and includes chapters on oats, wheat, rye, barley, rice, millets, sugar-cane, sorghum, and maize, prefaced by a chapter describing the general characters of the Gramineae. In each chapter the author describes the growth and development of the cereal under consideration, its detailed anatomy, and its genetics, and discusses the classification of the species and cultivated forms belonging to the genus, and also the origin of the cultivated kinds. At the end of each chapter there is an extensive bibliography including work published up to the end of 1935 and some of the 1936 publications, but, as the author remarks in his preface, "no attempt has been made to deal adequately with the literature of 1936". Considering the difficulty of obtaining certain literature in the Union of South Africa, these bibliographies are excellent, e.g. there are over 400 references listed at the end of the chapter on wheat and nearly 300 at the end of that on maize.

Vol. II includes the families containing the principal root, forage, fibre, and vegetable crops of the world. The longest chapters are those on the Malvaceae, Solanaceae, Leguminosae, and Cruciferae families, the other chapters dealing with the families Liliaceae, Moraceae, Polygonaceae, Chenopodiaceae, Linaceae, Umbelliferae, Convolvulaceae, Cucurbitaceae, and Compositeae. In his treatment of each family, the author describes the general characters very briefly, and follows this description by a classification of the genera and species of economic importance. He then describes the development, anatomy and genetics of the more important members of each genus and gives a brief account of those of lesser importance. The origin of the cultivated forms and their economic uses are also discussed. As in the volume on cereals each chapter is followed by an extensive bibliography. At the end of each volume there are very useful plant and author indexes and a very good general index. The books are well printed and profusely illustrated, most of the illustrations being copied from those of the original publications.

Agricultural botanists all over the world will be extremely grateful to Prof. Hector for these volumes, in which he has given a co-ordinated and masterly account of the immense amount of work which has been done since 1900 on the botany of cultivated plants. They will also be thankful for the references to the widely scattered literature on the subject. This work, also, has a much wider appeal than to agricultural botanists for the chapters on the Solanaceae, Cruciferae, and Chenopodiaceae families include not only plants that are usually grown on farms, but garden and market garden plants such as the tomato, egg plant, cabbages of all kinds, radish, sea kale, chard and spinach. In other chapters there are descriptions of onions, asparagus, melons, cucumbers, lettuce, artichokes, and other garden crops. The inclusion of these plants is of great value to the horticultural botanist for the literature on them is widely scattered and difficult to obtain. "Pure botanists" will also find these volumes very useful and they might with advantage use much of the material for courses in "pure botany" since our knowledge of many of the crop plants is far more detailed and extensive than that of many plants of theoretical interest which figure so largely in these courses. To the agricultural student who hopes to obtain a colonial post, the chapters on tropical and sub-tropical plants are most helpful.

The treatment of one or two of the families, particularly the Chenopodiaceae, is somewhat disappointing, the references to sugar-beet being distinctly out of date. Also, the author might with advantage have included descriptions of the external morphology and illustrations of many of the plants, instead of assuming the reader to have a knowledge of their appearance. These, however, are minor criticisms of a

work of this size, and Prof. Hector is to be congratulated on the production of a work of such a high standard of quality and usefulness.

ADELA G. ERITH.

Commercial Fruit and Vegetable Products; a Textbook for Student, Investigator and Manager. By W. V. CRUESS. 2nd Edition. Pp. x + 798. London: McGraw-Hill Publishing Co., Ltd. 1938. 36s. 0d.[1]

The first edition of this well-known American work was published in 1924. Since then important advances have been made in the technology and underlying sciences of the industries, the freezing storage of vegetables and fruits has attained industrial importance, and the prohibition law of the U.S.A. has been repealed.

Every chapter of the new edition shows emendation, the chapters on vitamins, canned-fruit spoilage, tomato products, and canning, have been completely revised, and there are additional new chapters dealing with plant pigments, enzymes of fruits and vegetables, freezing storage of fruits and vegetables, and the making of wines. The chapter bibliographies have in most cases been brought up-to-date. Ch. I dealing with microorganisms in relation to fruit and vegetable storage still needs some expert mycological attention.

The book is a comprehensive survey of an important and very wide field in which knowledge and application are advancing rapidly. The production of especially suitable varieties of fruits and vegetables for canning, drying, etc., is essentially a problem for the geneticist; pests and diseases in the growing·plants and harvested crops demand the entomologist and plant pathologist; the processes and conditions of preservation are problems for the plant physiologist; and problems of deterioration and spoilage are work for the mycologist and bacteriologist. The whole series of industries is based essentially on applied biology, and applied biologists should play a much larger part in these developments than they do at present, and they should receive from the industries a very much larger measure of support.

WILLIAM B. BRIERLEY.

The Rape of the Earth: A World Survey of Soil Erosion. By G. V. JACKS and R. O. WHYTE. Pp. 313 and 47 Plates. London: Faber and Faber, Ltd. 1939. 21s. 0d.

A few months ago the authors of this book published a short factual survey of soil erosion in various countries (see *Ann. appl. Biol.* 1938, **25**, 874) and they have now produced, under a strikingly apt title, a larger and more comprehensive work of quite outstanding importance.

Much of the world is, to a large extent, governed by or from Western Europe, and since in this region soil erosion is practically negligible, the subject has received little recognition. Only a few people have heard of it, and fewer still have any clear idea of its nature and importance; yet, as the authors state, "Soil erosion is altering the course of world history more radically than any war or revolution. Erosion is humbling mighty nations, re-shaping their domestic and external policies and once and for all it has barred the way to the El Dorado that a few years ago seemed almost within reach." Life on this earth depends upon a tenuous skin of soil, and over vast areas this skin is being attacked by "a contagious disease spreading destruction far and wide irrespective of private, county, state or national boundaries". "The only sure foundation upon which a superstructure of civilization can be built is a stable soil" and the very foundation of civilization and even of life itself is being blown into the air or washed into the sea. Of South Africa, General Smuts has said "Erosion is the biggest problem confronting the country, bigger than any politics" and this is equally true of many other vast regions.

In its catastrophic menace soil erosion is a thing of the last few decades and it is increasing at terrifying speed, but it is not an Act of God like an earthquake; it is

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[1] As from 2 October, 1939, the McGraw-Hill Publishing Co., Ltd. have raised the net prices of their books by 10 % throughout the whole of their catalogue.

man made and it can be stopped. "It is not so much the damage already done that matters as the final and inevitable disaster to civilization that will occur if the contagion is allowed to spread until it is uncontrollable. A war-scarred country can be restored to prosperity in a few years; a field stripped of its soil is finished, at least so far as providing for the living generation is concerned, but can continue to spread destruction over other land." "We now know fairly precisely what agricultural, pastoral, forest and engineering principles must be adopted to stop the earth from rotting away beneath our feet", but because of established conditions and practices "we cannot, or dare not, apply them forthwith on a scale commensurate with the gravity of the situation" save at the risk of "a social and political revolution". "Great changes will have to be made in the kinds and quantities of crops produced, exported and imported by different countries, internal and external trade relations and policies will be affected, and perhaps most significant of all, the conditions of land tenure and occupancy upon which the social structure of a civilized community is founded, will have to be re-defined. Erosion control, acknowledged in many rapidly developing countries as the most vital problem confronting them, means going back to the beginning and re-building human society in a frame whose shape is determined by the intrinsic nature of the soil and is independent of immediate economic or political considerations."

To many, perhaps most people, especially if they have not travelled in eroded lands, all this may seem wild exaggeration, but the authors support their statements with a wealth of data which, cumulatively, is almost overwhelming. Also, it may be objected, that large scale industrial processes of synthesizing food stuffs, or a huge development of water culture methods (Hydroponics) may, in the future, provide all the food that man requires and render soil farming unnecessary. But to many of us it would seem fairly clear that industrialism itself suffers increasingly from much the same kind of disease as does the soil, and it is very clear indeed that water culture methods have a long, long way to go before they can supply even a tithe of our food stuffs. In any case it would, from every point of view, be infinitely better to preserve our mother earth by controlling soil erosion and to use synthetic and hydroponically produced food stuffs as accessory. What *must* be recognized is that the soil is the world's most valuable natural resource, and that in formulating agricultural and social policies the rights of the land must be placed first.

The authors' approach is based on two simple theses which in essence are, first, that Western European civilization is based on an agriculture which has slowly evolved on the conquest of the forest in humid temperate regions and, secondly, that this agriculture, with all its concomitants, has been transferred to prairie, semi-arid and tropical regions to which it is proving quite unsuitable; erosion is one result of this unsuitability. "The agriculturalist's main object is to live *off* rather than *on* the land, to bleed his soil for the sake of distant towns, and himself to be maintained and enriched by their produce. Farming has become an industry and irreplaceable soil fertility an industrial commodity to be bought and sold and transferred to the ends of the earth as easily as any other commodity." "Cultivation of the soil became a means of wealth instead of a mode of life. The more that could be got out of the soil and the less that had to be put back, the better for the cultivator."

The book is a composite production, the more factual chapters being written by Dr Whyte, and the more speculative, i.e. economic, and social chapters by Dr Jacks, but the work coheres as a unity. Whilst one is not compelled to accept the detailed social and economic conclusions at which the authors arrive, probably every reader of this book will feel that they have pictured a condition of things the full and immediate recognition of which is vital to human life and welfare over vast regions of the globe, and that they have discussed the social and economic implications of this condition in an interesting and enlightening manner. All thinking men, not only in eroding countries but in Western Europe, whatever their political and economic views, must face up to this situation. The problem for which the last and present generation is largely responsible cannot be left for future generations to solve; we know what has to be done and it is imperative that we do it whilst it still remains possible.

WILLIAM B. BRIERLEY.

Science in Africa: A Review of Scientific Research relating to Tropical and Southern Africa. By E. B. WORTHINGTON. (Issued by the Committee of the African Research Survey under the auspices of the Royal Institute of International Affairs.) Pp. xiv + 746. London, New York and Toronto: Oxford University Press. 1938. 10s. 6d.

This volume forms part of the African Research Survey conducted under the general direction of Lord Hailey. Dr Worthington, who had contributed valuably to knowledge of the biology and fisheries of East African lakes, was asked to prepare a report on scientific research and technical services bearing on African conditions and development, and to this end visited Nigeria, Dahomey, Togoland, the Gold Coast, Sierra Leone, the Gambia, Senegal, and the French Sudan. He writes, therefore, with unusual first-hand knowledge of African conditions.

The book covers an enormously wide field, the several chapters dealing with general problems of research, surveys and maps, geology, meteorology, soil science, botany, forestry, zoology, fisheries, entomology, general agriculture, crop-plants, plant industry, animal industry, general health and medicine, human diseases, health and population, and anthropology. The author does not pretend to knowledge or opinion outside his own study or experience, but simply states the views of experts as objectively as possible without attempting to assess the value of rival theories. The material is arranged more or less uniformly in each chapter under the headings of introduction, organization, and results, and territorial arrangement is followed when subjects lend themselves to such treatment. In consequence, although the book contains an enormous mass of data, it is extremely easy to use. The more important research up to 1936 is included, with the exception of medical research which is more or less complete only to the end of 1934. The various chapters of the volume in draft were submitted for criticism and suggestion to numerous specialists listed on pp. 615–25, but the volume is essentially the personal achievement of Dr Worthington. The sources of information are given in a classified and extremely useful bibliography occupying pp. 627–91, and there is an excellent index.

It is impossible in a brief notice of a volume so wide in its range to enter into any detail, or in any way to do justice to the quality of the work. Browsing through the volume and reading critically chapters and sections dealing with subjects in which one is particularly interested, one is impressed not only by the wide scope and comprehensiveness of the work, but by the balance and fairness of the author's treatment, and most of all, perhaps, by the insight he shows into problems and research outside his own special knowledge and experience. The book gives one a remarkably clear picture of the state of scientific research in Africa, of its organization, of the problems under investigation and of those partially or wholly solved, of the infinite number of problems to be faced, of the way in which the problems integrate with each other, and of the difficulties created by their interdependence. At the same time the book is no dry-as-dust catalogue; Dr Worthington writes well and his book is extremely interesting and readable, being at once both informative and suggestive.

As the author states, "A development based on a real understanding of Africa's potentialities has hardly yet begun, and will be impossible until the necessity of scientific knowledge is recognised." Few books could do as much to bring about this recognition as *Science in Africa*, and one can only be grateful for Dr Worthington's achievement, for there are very few scientific workers who would have had the courage to assay his task or to complete it so successfully.

Finally, one may perhaps draw attention to the fact that through the generosity of the Carnegie Corporation of New York, this beautifully produced volume of 746 densely packed pages, illustrated by eight plates, four text-figure maps, and a large coloured folding map with insets, is obtainable at the remarkably low price of 10s. 6d.

WILLIAM B. BRIERLEY.

Health and Nutrition in India. By N. GANGULEE. Pp. vii + 337. London: Faber and Faber, Ltd. 1939. 15s. 0d.

Dr Gangulee was formerly Professor of Agriculture and Rural Economy in the University of Calcutta, and served as a Member of the Royal Commission on Indian Agriculture. The thesis of his book is that India's main problem is one of health and nutrition in relation to socio-economic conditions, and that the limiting factors are poverty and shortage of food. The problem is first stated in general terms, and then the necessary basis for its consideration is given in an account of modern nutritional science and the consequences of dietary deficiencies. The conditions of public health in India are then described with special reference to the main theme, and a survey is made of Indian foodstuffs, and of the diets of various Indian communities. These six chapters thus describe the general facts and principles and their exemplification in India. The author then discusses what is being done in certain other countries with regard to analogous situations and, finally, considers the problem in India and makes concrete suggestions for action.

In the factual chapters the author is admirably clear and concise—chapters II and III, for example, contain one of the best simple accounts I know of the principles of modern nutritional science. But Prof. Gangulee's volume is not a textbook of dietetics; it is a thoughtful and constructive essay on a major human problem, the mental and physical health of nearly 400 million people. And in writing of this tragedy Prof. Gangulee is not merely a scientist writing of matters of fact but a man writing of his own country and of his own people. His pen distils a rich humanity, a sustained enthusiasm, and a flame of idealism tempered only by clarity of vision, unusually wide knowledge, and a wealth of sobering experience. It is not possible here to give any idea of the author's treatment of his subject, or of the richness of his book in scientific data, constructive thought, and practical suggestion. The book is an essay in applied biology, applied biology on an epic scale, and the author shows clearly how the application of biological knowledge we already possess could ameliorate the lives of one-fifth of the world's population. No-one who cares for India dare neglect this courageous book.

WILLIAM B. BRIERLEY.

An Introduction to Modern Genetics. By C. H. WADDINGTON. Pp. 441. London: George Allen and Unwin, Ltd. 1939. 18s. 0d.

Dr Waddington's own researches have lain in the field of experimental embryology rather than genetics but, in many ways, this adds to the value and interest of his work since he is able to view data and relations from a wider angle than seems to be possible by most professional geneticists. His aim is not so much merely to add another formal textbook of genetics to the library already in existence as to integrate genetics with organic development and evolution, a more useful and difficult task. As he says in his Preface: "I want to urge that the connection between genetics and the other branches of biology, such as cytology, embryology, the study of evolution and of the biochemical nature of cell constituents,' is much closer than is often admitted, and that the boundaries between these subjects deserve less attention than is usually paid to them."

The subject-matter of the book is divided into five parts. Part I deals with formal genetics—the fundamentals of Mendelism, modifications of the chromosome cycle, the behaviour of individual chromosomes, their linear differentiation, and their mechanics. All this forms the modern groundwork of the subject and occupies about one-third of the book. As a brief presentation of the general situation to-day these chapters seem to me quite excellent, but since schools of thought are now growing up in genetics there will almost certainly be geneticists who will find grounds for cavil with both the detail and the balance and perspective of the author's treatment. Incidentally, the brief summary on p. 27 does not correspond with the actual chapters.

Part II deals with genetics and development and contains chapters on genes and development, the interaction of genes and its effects, gene controlled processes, the genetic control of pattern, and sex determination. It is in this part especially that the author's developmental viewpoint and his wide range of knowledge have full scope, and his consideration of these difficult problems is illuminating.

Part III is devoted to genetics and evolution, contains an introductory chapter on the processes of evolution, and then two stimulating if somewhat controversial chapters on the genetic nature of taxonomic differences and evolutionary mechanisms. The relation of cytogenetics to organic evolution is often either evaded by genetical writers or dealt with quite inadequately, and Dr Waddington's brief but masterly discussion of this problem is, therefore, all the more welcome.

Part IV which deals with genetics and human affairs contains only two chapters. The first is a very summary account of animal and plant breeding and, although one sympathizes with the author's difficulty in spelling "*Phytophthora*", this chapter seems to me far below the general standard of the book. The chapter on human genetics is, however, quite brilliant and one can only wish that some of the more rabid eugenists and racialists could be induced to read it.

To more general readers Parts III and IV will be by far the most interesting in the book. In Part V the author returns again to more abstruse matters and discusses the nature of the gene. Goldschmidt's somewhat pessimistic views are regarded as "extremely premature" and the numerous alternative hypotheses are clearly and fairly stated.

A useful Appendix describes laboratory methods for class work on *Drosophila*. The book opens with a synoptic contents and concludes with an excellent combined bibliography and author index (which contains a number of misprints) and an inadequate subject index. It is illustrated by 5 plates and 160 text-figures, many of the latter being over reduced, and it is bound in a very startling cover.

The author writes in a condensed but clear and interesting style and, although for general scientific readers the book will not always be easy going, the effort will be found well worth while. Not only in all fields of biology but in the more general work-a-day world the data and implications of modern genetics are so vitally important that it is to be hoped that this book will reach a very wide public. Not only is it remarkably up-to-date but it seems to me the best introduction to genetics in its wider biological relations that has yet been published.

WILLIAM B. BRIERLEY.

Earth's Green Mantle. By S. MANGHAM. Pp. 332. London: The English Universities Press, Ltd. 1939. 10*s.* 6*d.*

This is an excellent and unusually comprehensive survey of modern plant science written for the general reader. Although the author's choice of material and points of view are essentially those of a teacher of botany in an academic institution he shows considerable sympathy with the more applied aspects and it is interesting that in his final chapter, headed "Progress and Prospects", his viewpoints and material are entirely applied. The book opens with an effusive foreword by Sir Arthur W. Hill, and closes with a somewhat unsatisfactory list of books suggested for further reading, and an Index. It is illustrated by forty-two text-figures and forty plates, each of the latter containing from one to eight excellent photographs which, in many cases, suffer from over-reduction.

The book is accurate, up-to-date, and interesting, for the author has performed his task well. It is written in a non-technical but rather pedantic style with a tendency to the use of such language as "excavatory operations", "the revelations of this optical servant of the scientist", "the resources available for probing life's mysteries", and even "How prodigal Nature is!"

WILLIAM B. BRIERLEY.

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